CODED AMINO ACIDS : AN EVOLUTIONARY LOOK WITH SYNTHETIC PERCEPTION

A Thesis Submitted
In Partial Fulfilment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

by

WAHE GURU PAL SINGH

to the

DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY, KANPUR
JANUARY, 1988

Dedicated to

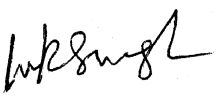
the memories

of my father

STATEMENT

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the Department of Chemistry, Indian Institute of Technology, Kanpur, India, under the supervision of Professor S. Ranganathan.

In keeping with the general practice of reporting scientific observations due acknowledgements have been made wherever the work embodied is based on the findings of other investigators.



Waheguru Pal Singh

CERTIFICATE

Certified that the work contained in this thesis, entitled, **"CODED AMINO ACIDS: AN EVOLUTIONARY LOOK WITH SYNTHETIC PERCEPTION"** has been carried out by Mr. Waheguru Pal Singh under my supervision and the same has not been submitted elsewhere for a degree.

Kanpur
January , 1988

(S. Ranganathan)
Thesis Supervisor

DEPARTMENT OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY, KANPUR, INDIA

CERTIFICATE OF COURSE WORK

This is to certify that Mr. Waheguru Pal Singh has satisfactorily completed all the course requirements for the Ph.D. degree programme. The courses include:

Chem. 502	Advanced Organic Chemistry
Chem. 505	Principles of Organic Chemistry
Chem. 524	Modern Physical Methods
Chem. 525	Principles of Physical Chemistry
Chem. 545	Principles of Inorganic Chemistry
Chem. 581	Basic Biological Chemistry
Chem. 801	Graduate Seminar
Chem. 800	General Seminar
Chem. 900	Research

Mr. Waheguru Pal Singh has successfully completed his qualifying examination in July, 1983.

(P.S. GOEL)
Professor and Head
Department of Chemistry

(S. SARKAR)
Convenor
Department of Post
Graduate Committee

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Master Anand has always been an integrated part of my stay in the laboratory with his intelligent and humorous nature.

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Waheguru Pal Singh

PREFACE

ORGANIZATION

The thesis entitled **"CODED AMINO ACIDS: AN EVOLUTIONARY LOOK WITH SYNTHETIC PERCEPTION"** consists of six parts namely, a. Introduction, b. Background, c. Present work, d. Spectra, e. Experimental and f. References.

SUMMARY OF THE PRESENT WORK

The focus of work incorporated in this thesis is directed towards the possible understanding of rationale pertaining to the selection of the twenty coded amino acids from amongst the infinite variety possible and from over 500 such structures that exists in nature. Such an understanding could not only provide an insight relating to processes directed at optimization of the functional system, but also that, the understanding gained from these studies could find practical applications in in-vitro circumstances.

The relationship between the functional system and the information system, as dictated by the genetic code, is maintained by species separated by even vast evolutionary gaps. The code, made up of 64 triplets and connected to the choice of the twenty coded amino acids, has a non randon profile that could be exemplified with the extreme cases of tryptophan and

methionine which have a unique code on the one hand and leucine, serine and arginine which have a degeneracy of six on the other. These non random degeneracies could in principle be fitted into a framework of evolution, wherein opportunities are provided for more appropriate α -amino acid residues, as a function of lifting of the degeneracy.

Even today seven of the twenty coded amino acids have a defacto doublet code in the sense that the third letter is unimportant in the selection of that particular amino acid. Perhaps from the evolutionary point of view the most interesting are the five pairs of amino acids, namely, phenylalanine-leucine, histidine-glutamine, lysine-asparagine, aspartic acid-glutamic acid and serine-arginine. These share a common doublet with the last letter assigned to a pyrimidine code for one partner and a purine for the other.

That this special relationship amongst the five pairs is not a fortuitous occurence is brought out by an analysis of the relative frequencies of coded amino acids observed in over 1500 closely related proteins which brings out the fact that there is an overwhelming prefence for mutations that would result in the interchange of these partners with any other. Apart from the special attributes pertaining to the above five pairs, they have similar structural profiles, which, as in the case of aspartic acid-glutamic acid pair is obvious but in the case of glutamine-histidine rather subtle. Consequently, from the point of view of

the genesis of these pairs into the coded system as well as from synthetic point of view the mutual transformation of these pairs was considered of interest and the glutamine-histidine pair has been chosen in the present study.

On the basis of an antithetic analysis the generation of the twenty different α-amino acid structures could be considered as an aggregate of 3 structural elements; a. the generally invariant peptide backbone that carries the chiral centre and contributes to the secondary structure, b. the methylene spacer which is crucial to the precise structure of the protein and c. the end group involved in polar and hydrophobic interactions.

The present work has endeavoured to demonstrate that the spacer methylene plays a pivotal role in the makeup of the functional system. Thus, the present work has demonstrated that spacing of the basic amino group from the peptide backbone by as many as 4 methylenes are essential for incorporation in to the coded system and that pairs having the same functional group and whuch differ in as little as 1 spacer methylene could exhibit very divergent profiles in reactivity.

Another remarkable feature associated with the functional system, is that with the genetic code coming into vogue, in structural proteins like collagen, the possible inadequacies pertaining to maintenance of proper structure with a normal complement of coded amino acids, as dictated by the code, are overcome via very remarkable post translational operations.

This can be exemplified with proline and lysine which are tranformed to respectively 4-hydroxyproline and δ-hydroxylysine, in each case involving the functionalisation of an inactive position. Endeavours to chemically simulate the remarkable lysine to δ-hydroxylysine post translational modification in proteins via attaching a pendant unit aimed at effecting the desired functinalisation via a six membered transition state followed by removal of the pendant has been explored in the present work.

The transformations of chain shortened lysine anologs generated from L-glutamine and L-asparagine.

The spontaneous cyclization of the chains shortened analogs of lysine generated from N-protected glutamine esters coupled with the demonstrated stability of this unit in a peptide environment provide not only experimental proof to the effect that chain shortened lysine analogs cannot be supported on t-RNA but also that, were such units be generated post-translationally, they can be present as viable side chains in proteins. The overwhelming preference for intramolecular cyclization of the lower homologs of lysine esters would explain a unique feature of this amino acid namely that is comparison with the other 19 coded amino acids the functional group of lysine is held 4-methylenes away from the peptide backbone.

These conclusions were arrived at from studies with N-benzyloxycarbonyl glutamine esters (1)*, and C-protected dipeptides having N-benzyloxycarbonyl glutamine residue (2), as the substrates and via using PhI $(TFA)_2$ as the reagent to bring about the desired $CH_2CH_2CONH_2 \rightarrow CH_2CH_2NH_2$ change. The reaction of N-benzyloxycarbonyl glutamine esters (1a), (1b) and (1c) with $PhI(TFA)_2$ in $CH_3CN:H_2O::1:1$, at rt for 3h resulted in the expected $CONH_2$ to NH_2 degradation and when these were left stirred with aq. $NaHCO_3/CH_2Cl_2$ for 3h, underwent spontaneous cyclization in each case giving rise to 3-N-benzyloxycarbonyl pyrolidine 2-one (3) in, respectively, 55%, 70% and 45% yields.

The glutamine side chain of peptides (2a), (2b) and (2c) were effectively degraded with the reagent in aqueous DMF and the resulting amino compounds (4a) and (4b) were isolated and fully characterised . Endeavours, however, to demonstrate the side chain mediated rupture of the peptide bond in (4a), (4b) and (4c) that would have resulted in the formation of the lactam (3) under conditions of the (1)→ (3) change as well as others failed. Thus, in sharp contrast to the instability of $NH_2CH_2CH_2CH(NHZ)COOR$, (4) was recovered in each case.

The $H_2NCH_2CH(NHZ)COOMe$ (6) from the $PhI(TFA)_2$ mediated degradation of $H_2NCOCH_2CH(NHZ)COOMe$ (5) was equally unstable.

These numbers refer to those assigned in the present work (SECTION. C AND SECTION. E)

Neither the β-lactam corresponding to (3 nor any other pure product could be isolated under conditions of the (1)→ (3) change. That the expected product (6) did form, was demonstrated via reaction with PhNCS leading to the thiourea (7b) in 63% yields. The expected cyclisation reminiscent of the (1)→ (3) change could be demonstrated even with (6) when it is reacted with an appropriate helper molecule. Thus, the reaction of (6) with ethylisothiocyanate gave the novel cyclised product (8), 20% and the thiourea (7a), 41%. It was further shown that the thiourea (7a) cyclizes to (8) in refluxing benzene . The chiral 5, 6-dihydro uracil from the above cyclisation is interesting in the sense that it can be quite easily made, provides, in principle, opportunities for the preparation of diversely substituted analogs by use of an appropriate isothiocynate and, finally, the thioamide unit present should enable the selective attachment of sugar residues to yield novel nucleosides and nucleotides.

Finally, in striking contrast to the degradation of the glutamine dipeptides, the asparagine analog $H_2NCOCH_2CH(NHZ)CONHCHCH_2PhCOOMe$ (9), under the same conditions was hardly touched. This profound difference in reactivity is fortunate since it would enable the specific degradation of glutamine side chain in peptides without affecting asparagine residues that would have lead to complications arising from the presence of the unstable NH_2CH_2 unit.

The present work has shown that the α - aminoethyl analogs derived from the coded amino acid glutamine cannot be used for translation because of great propensity for cyclisation. The glutamine to the corresponding α - aminoethyl side chain modification can however be brought about smoothly in peptides leading to the synthesis of a variety of potentially useful modified proteins. In a peptide environment the glutamine side chain can be selectively degraded over its lower homolog asparagine.

Studies on the transformation of glutamine → histidine in a peptide environment.

The special relationship between the histidine - glutamine pair in the coding system has already been mentioned. On their own right this pair plays perhaps the most crucial role in life systems than in any other. Histidine is present in the active sites of almost all enzymes and glutamine is critical for nitrogen metabolism. Although the structural similarity between histidine and glutamine is not that apparent, a retrosynthetic analysis could quickly show that the addition of elements of formamide to glutamine, followed by cyclisation, could lead to histidine. In the present work the transformation of the glutamine side chain to that of histidine presented an attractive objective. It was considered practical to degrade glutamine to a lower nitrile and then to generate the imidazole ring via cycloaddition to an N-methyl formamide equivalent. The proposed plan required the transformation of glutamine to β -

cyanoalanine followed by cycloaddition with a conjugate base of arylthiomethyl isocyanide.

N-benzyloxycarbonyl glutamine methyl ester (1a) was degraded with $PhI(TFA)_2$ as described earlier, and than directly oxidised to N-benzyloxycarbonyl β -cyano alanine methyl ester (10). This facile transformation not only accomplishes the first stage in the glutamine → histidine transformation but also represents a novel method for transformation of an amide to its lower nitrile.

The subsequent efforts called for a cycloaddition involving the rather unreactive nitrile function which wsa sought to be accomplished with conjugate bases of arylthiomethyl isocyanides. Such cycloadditions would lead to thioaryl imidazoles that could be desulfurised to the desired objective. At the outset, in order to overcome the major problems associated with arylthiomethyl isocyanides used for such cycloaddition reactions, namely, their pervasive and highly disagreeable odor, and the inability to have then prepared in pure state in quantities, a novel approach had to be employed, not only with reference to the arylthiomethyl isocyanide itself, but also the methodology for preparing it.

The preparation of the reagent of choice namely 2-thionaphthylmethyl isocyanide was envisaged via the transfer of a formamidomethyl unit to 2-thionaphthol. The needed transfer reagent N-(Formamidomethyl)N-benzyl morpholinium iodide (12) was prepared in an over all yield of 64% from morpholine, and the

transfer effected in 90% yields via reaction in dry benzene in the presence of triethylamine. The resulting 2-thionaphthylmethyl formamide (13) was converted to (11) in 80% yields using triphenylphosphene, CCl_4, $CHCl_3$ and Et_3N. The present procedure for (11) is most attractive. This isocyanide is totally devoid of the highly disagreeable and pervasive odor associated with the earlier isocyanides.

Although formamidomethyl transfer to sulfur has played a very important role in synthetic organic chemistry, particularly in the preparation of methylthio isocyanides, the mechanism by which this transfer takes place has not been investigated. Rather surprisingly, such experiments with the transfer synthon reported in the present work has shown that the transfer most probably takes place via a fragmentation recombination mechanism and the process is complicated by equilibrating intermediates. Such an integrated mechanism was able to rationally explain the ability of the transfer reagent to effect the formamidomethyl transfer to some nucleophiles and to give an unexpected product involving other nucleophilic centres.

The efficacy of 2-thionaphthylmethyl isocyanide for the transfer of elements of CH_3NC has been established via cycloaddition of conjugate base generated in n-BuLi at -78° C to CH_3CN and PhCN leading to respectively 4-thionaphthyl 5 methyl imidazole (20) and 4-thionaphthyl 5 (4) phenyl imidazole (21) in 95% and 64% yields. These adducts suffer from preferential

shielding of the two possible nitrogens and could find use in preparation of specifically N-protected imidazole.

Compounds (20) and (21) were smoothly desulfurised to the corresponding 4, 5 methyl imidazole (22) (76%) and 4, 5 phenyl imidazole (23) (56%) using freshlly prepared W-6 Raney nickel. Repeated endeavours to translate the practical imidazole synthesis developed to effect cyclo addition of the conjugate base of (11) to Z-alaβ-cyano-OMe (10) obtained earlier from glutamine, that would have eventually lead to the desired N-benzyloxycarbonyl histidine methyl ester did not succeed. These endeavours included diverse reaction conditions and attempts to enhance the efficiency of the reagent towards cycloaddition. In some cases evidences were obtained for presence of the desired imidazole but no pure product could be isolated from such experiments.

Studies on chemical simulation of post translational lysine hydroxylation.

The specific δ-hydroxylation of lysine in collagen, which is present to the extent of about 4%, reflect factors associated with agents such as enhanced crosslinking and shrinkage. The mechanism of this unusual reaction is not very clearly understood. Although the presence of glutaric monoperacid is implicated. In the present work, as a working model, endeavours were made to chemically simulate such hydroxylation via covalent

linkage of the ω - amino group to a quinazoline moiety and effect the functionalization through a 6 membered transition state. The quinazoline moiety is ideally suited, for removal after the functionalization has been achieved.

The rather interesting quinazoline ω -lysine-copper complex (Q-Lys-Cu) (26) was obtained in a single reaction via treatment of the lysine copper complex with 4-chloroquinazoline in 82% yields. The N-pretection of (26) was accomplished by appropriate methods leading to N-Z- ω -Q-Lys (27) and N-Bz- ω -Q-Lys (28).

Surprisingly, the C-protection of these compounds sought via reaction with diazomethane, resulted in N-methylation as well, leading to in the case of (27) to the corresponding dimethyl compound (29). Compound (29) represents a situation where 2 methyl quinazoline unit is specifically attached to ω -amino function of lysine in a peptide environment. Efforts to obtain N-C-protected Q-Lys without the complexity of ring methylation did not succeed . Very preliminary studies seem to suggest that oxigenation in presence of carriers like Fe^{+3} tend to promote N-oxide formation of quinazoline moiety eventually leading to its facile detachment.

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SECTION A. INTRODUCTION

Latent in the manifestation of every one of the metabolic processes that take place in nature, are endeavours spanning millenia, to optimise and to integrate chemical transformations and tune to maximum efficiency. More than in any other domain, this aspect is highlighted in the functional system, comprising of 20 coded amino acids, which via myriads of constellations can bring about a plethora of chemical transformations. The present work is aimed at the understanding of processes related to the choice of the 20 coded amino acids amongst the many possible on the one hand, and to chemically simulate post translational side chain modifications involving functionalization of inactive sites on the other.

The introduction of the tools of organic chemistry to understand, appreciate, and to solve problems, all related to proteins at the outset deployed with great trepidation, has, as a function of time, evolved into a dominant part of activities in this arena. This is best reflected in contemporary capabilities pertaining to breaking of protein chains, taking advantage of the individual characteristics of the side chains of coded amino acids. An account of accomplishments in this domain is considered as an appropriate background for the present work and comprises of section B.

SECTION B. BACKGROUND

The rupture of proteins at specific sites has played a very important role in the structural elucidation of such molecules. Additionally, in recent years, with the recognition that long chain proteins could best be synthesized by restructuring fragments, the necessity to develop methodologies that would break the peptide backbone at specific sites, selectively, cleanly, in good yields and under mild conditions has become paramount. The efficiency of peptide rupture was perhas not a very important criterion when used for structural elucidation and indeed many of the methods that have become in vogue, do not necessarily meet this requirement. However, with the potential use of such methods for protein synthesis, the yields obtained and the efficiency with which the reaction takes place have become crucial. The methodologies available for protien rupture in the literature form the background to the present work[1]. They are presented in a novel manner with the 20 coded amino acids arranged alphabetically and the methodologies available for specific rupture involving one of these , either singly, or pairwise, make the format of the presentation. Additionally the mode by which the side chain effects the cleavage is indicated by an arrow denoting whether it is from the N-terminal end or the C-terminal direction. The versatility of the chemical procedures to bring about protein rupture at specific sites is graphically presented in CHART-1-B wherein the 20 coded amino acids are arranged according to their relationship with the information

CHART-I-B

PHE	SER	TYR	CYS
LEU		TERM	TERM
			TRP
LEU	PRO	HIS	ARG
		GLN	
ILEU	THR	ASN	SER
MET		LYS	ARG
VAL	ALA	ASP	GLY
		GLU	

CHART - II-B

ASN → GLY

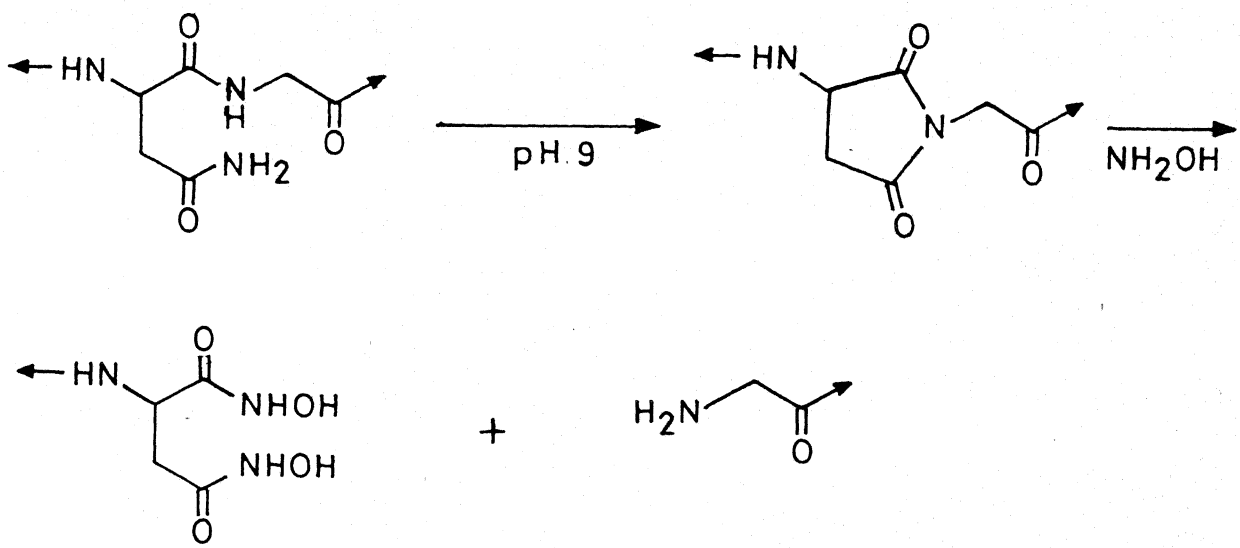
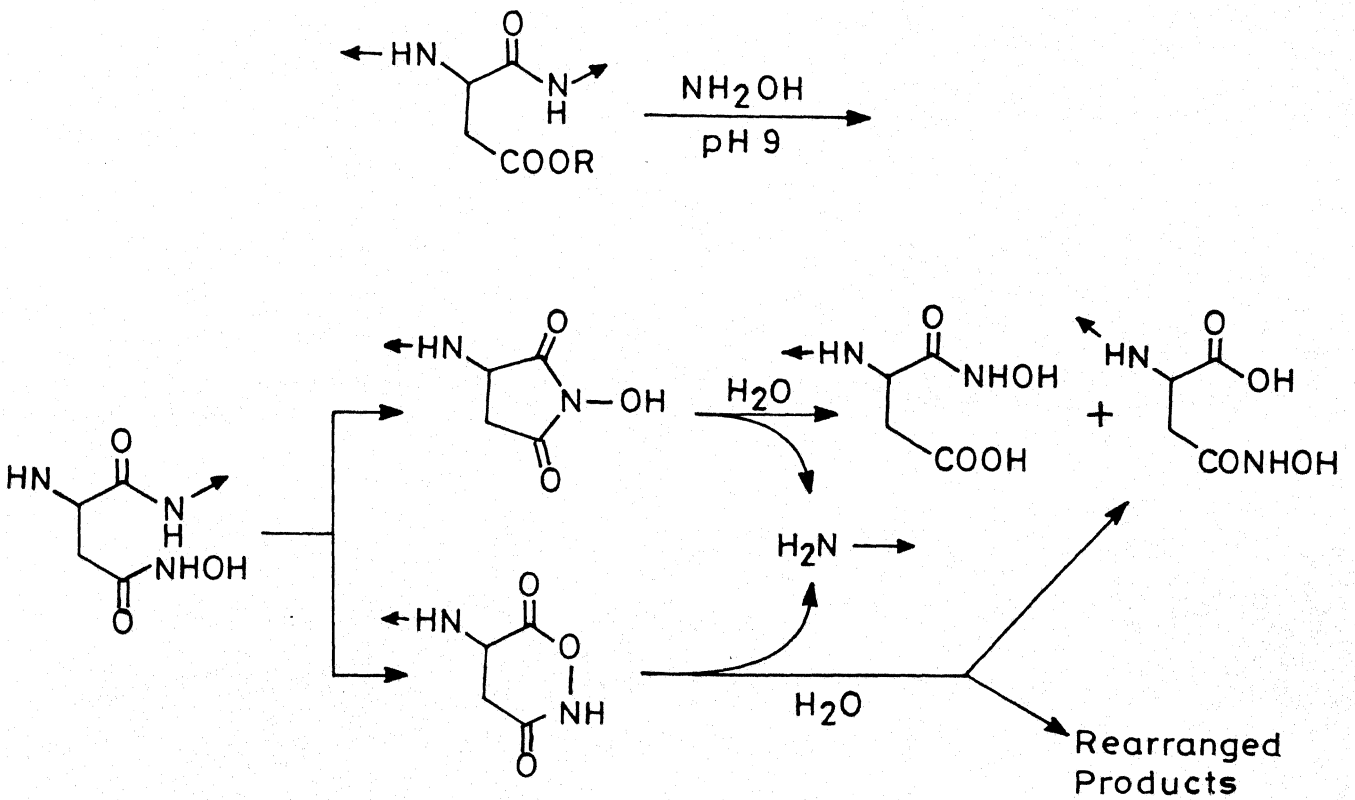


CHART - III-B

ASP →



system. It could be seen from CHART-1-B that as many as 12 side chains can be made as markers for proximate peptide rupture. The chemical tools available then, would play a pivotal role in the structural elucidation of unknown proteins and in the effecient synthesis of long protein chains by restructuring strategies.

ASPARAGINE→ GLYCINE

The asparagine-glycine pair enjoys a special relationship in the sense that at pH-9 and in the presence of hydroxylamine the asparagine moiety brings about the peptide bond rupture, as shown in CHART-2-B[2,3,4]. The mechanism involves the loss of ammonia from asparagine via an intramolecular nucleophilic attack of the adjacent peptide bond and the succinimide thus formed readily undergoes opening with hydroxylamine leading to peptide cleavage.

ASPARTIC ACID→

The fact that the type of cleavage described above could take place with aspartic acid-β-methyl ester adjacent to a glycine residue in presence of hydroxylamine has led to a very detailed study pertaining to the mechanism of this reaction. A variety of peptides of the type NAc Asp (β-NHOH)-X were prepared and their cleavage examined kinetically as a function of pH and temperature of the medium. These studies have shown that the hydroxamic acids arising from the reaction of aspartic acid-β-methyl ester and hydroxylmine can indeed bring about the rupture of the succeeding peptide bond irrespective of the nature of the

partner. This valuable result would mean that the mechanism of the unique asparagine-glycine bond breakage with hydroxylmine, which undoubtedly is strongly influenced by steric factors, is different from the cleavage involving the above hydroxamic acids. The peptide rupture here occurs largely by two pathways, as shown in CHART-III-B[5], where the nucleophilic sites available in the NH-OH moiety, namely, the nitrogen as well as the oxygen, are deployed to bring about the peptide cleavage, leading to, respectively, an N-hydroxy succinimide intermediate and a 6-membered oxaza system. In both cases the amino end of the ruptured peptide is liberated. The reactive intermediates thus formed readily undergo hydrolysis leading to open systems as shown in CHART-III-B at the carboxyl end. It should be noted that in the case of asparagine, the peptide nitrogen initiates the process of cleavage whereas in the case of the hydroxamic acids the cleavage is initiated by the side chain. This would account for the profound differences with respect to steric factors involved in the transition state. The hydroxamic acid method has been used to cleave a very large number of dipetides proximate to the aspartic acid residue. The reaction is promoted by added hydroxylamine. A comparison of the efficiency of the asparagine-glycine cleavage with that of NAc Asp (β -NHOH)-X peptide rupture is presented in TABLE-1-B. This clearly shows that the hydroxamic acids are not only more versatile in the sense that it cleaves any proximate peptide bond, but also the efficiency with which this reaction takes places is very superior.

TABLE - I-B

MAXIMUM % CLEAVAGE

	NH_2OH		NO NH_2OH	
	pH 8–9	pH 6.4	pH 9-10	pH 6-7
NAC ASP (NHOH) ALA	100	96	65	57
NAC ASP (NHOH) GLY	—	84	16	53
NAC ASN - GLY	42	84	0	0

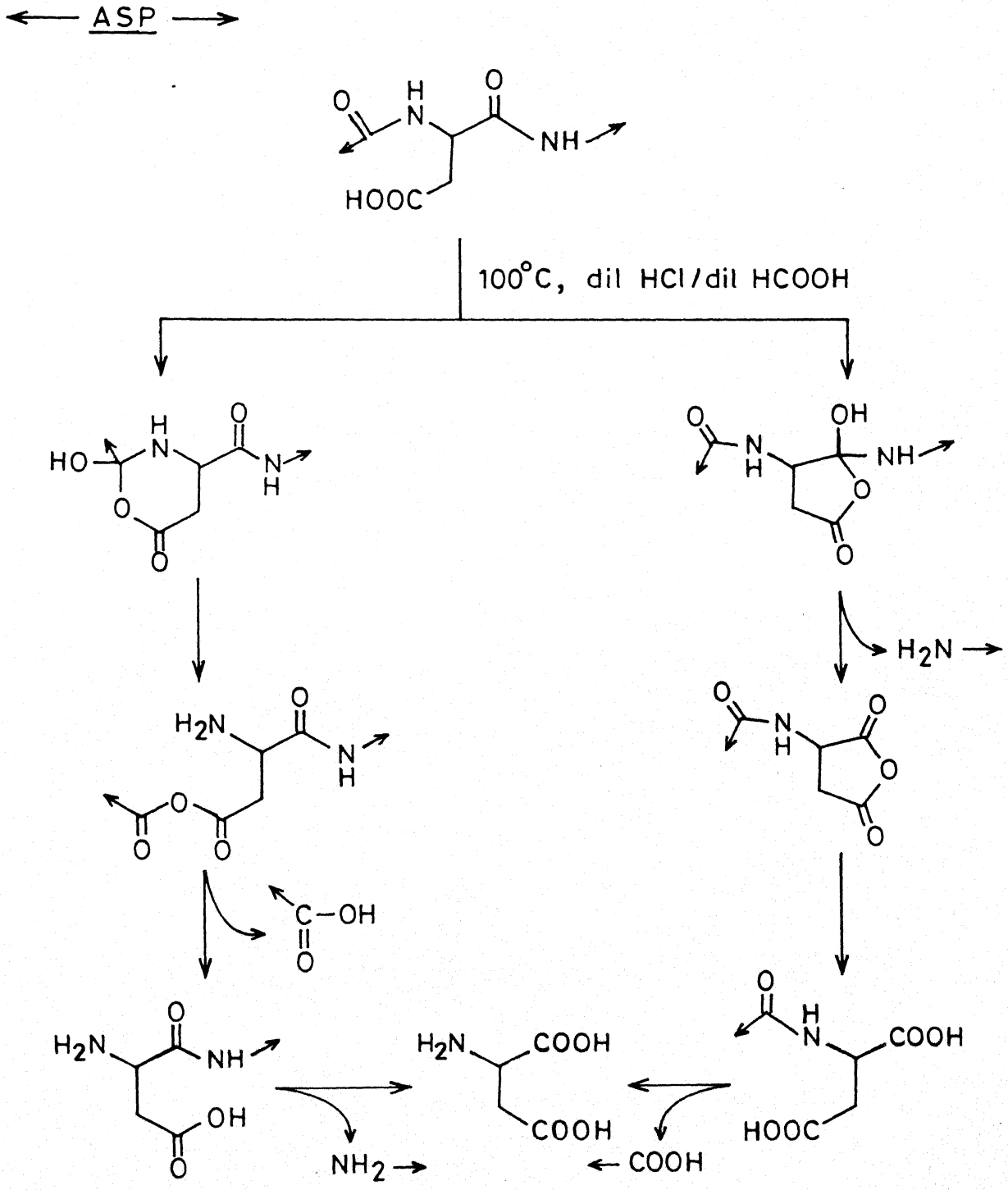
←ASPARTIC ACID →

In the illustrations cited relating to the capabilities of a coded amine acid side chain to bring about specific cleavage, the side chain responsible largely became part of the C-terminal of the ruptured peptide, either, as such, or with functional group modification. The example where the break can occur from both ends, leading to the liberation of the free amino acid, is that of aspartic acid. It has been shown recently that this residue in dilute HCl or in dilute formic acid, at 100°C in vacuo brings about the rupture of the peptide bond from both sides leading to the liberation of aspartic acid CHART-IV-B[6]. That this reaction is quite efficient, has been demonstrated by the digestion of the enzyme apovitellenin where the Asp_{16}-Ala_{17} was largely cleaved and the Asp_{39}-Ala_{40} cleaved to a very large extent. The same sort of results were obtained from studies on cytochrome-C. A remarkable observation was that asparagine is not able to bring about such a cleavage. The mechanism of this reaction is presented in CHART-IV-B.

ASPARTIC ACID/GLUTAMIC ACID→

The transformation of either aspartic acid or glutamic acid to homoserine or bishomoserine by reduction with either lithium borohydride or diborane in THF, followed by treatment with acid in the pH range of 1-2, leads to the cleavage of the proximate peptide bond. The methodology needs further experimentation in

CHART - IV - B



the sense that the reduction has to be made more efficient and the conditions of the cleavage made less stringent. (CHART-V-B[7,8]).

ASPARTIC ACID→ PROLINE

The aspartyl-proline peptide bond selectively splits under mild conditions at 40° C in aq. acetic acid adjusted to pH 2.5 with pyridine-guanidine hydrochloride. The ease with which this reaction takes place highlights the highly susceptible nature of proline peptides, possessing a tertiary nitrogen (CHART-VI-B[9]).

ASPARTIC ACID → PROLINE

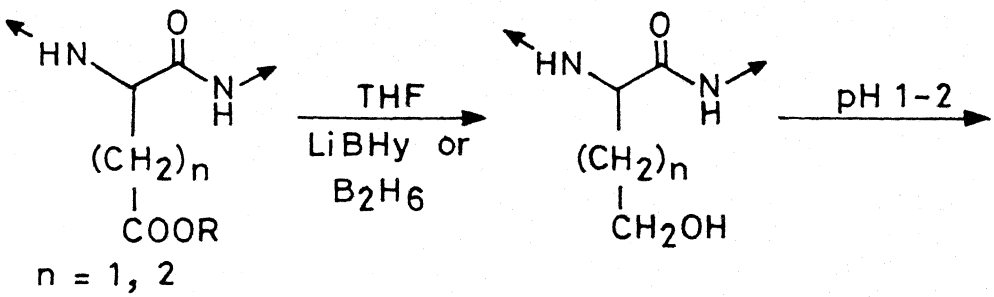
The reaction of concentrated acids brings about the rupture of the peptide chain at the aspartic acid-proline site (CHART-VII-B[10]). The prolyl peptide - a tertiary amide, undergoes breakage by intramolecular cyclization of the side chain of the aspartic acid. Although this method has been used to make larage fragments from complex proteins, the requirement of a strong mineral acid and the fact that the Asp-Pro proximate relationship is quite uncommon, makes this methodology of peptide rupture quite insignificant.

←CYSTEINE

Methodologies to cleave peptide bonds that are proximate to

CHART - V-B

ASP/GLU ⟶



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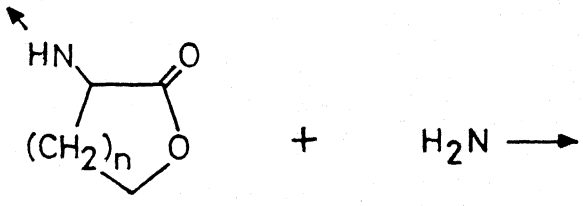
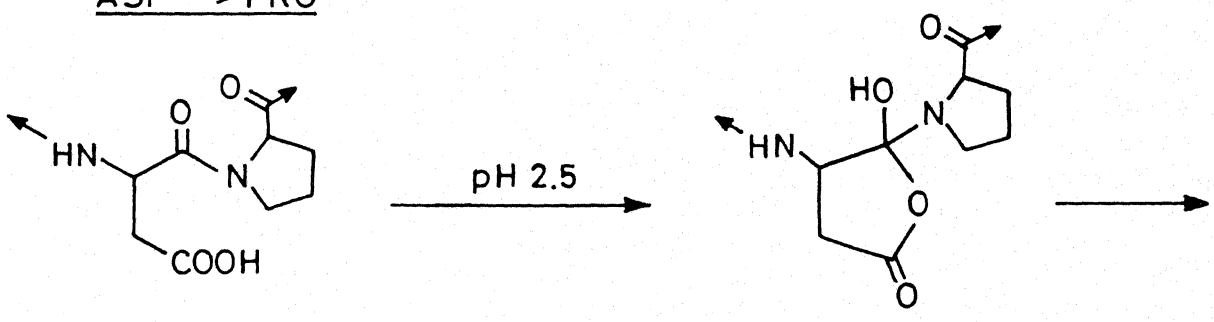
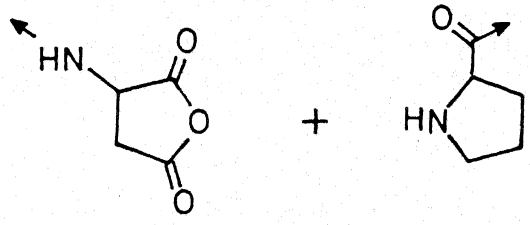
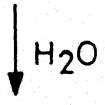


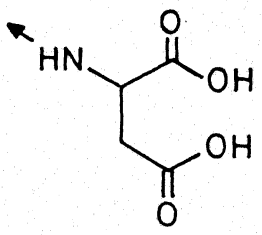
CHART-VI-B

ASP ⟶ PRO









a cysteine are of importance in the structural elucidation of complex proteins. Cysteine residues are always present as the oxidized cystine enabling the protein to have highly specific structures. The mild reductive cleavage of cystine to cysteine and subsequent cleavage, would mark the sites of linkages. The change of the cysteine SH to a thiocyanate by accepting equivalent of CN^+, leads to the peptide rupture from the carboxyl end via nucleophilic addition to the highly electrophilic cyano function CHART- VIII-B[11,12]. Paranthetically, this methodology constitutes one of the very rare examples where the peptide bond, through the nitrogen, behaves as a nucleophilic centre. As could be seen from CHART-VIII-B, the N-terminal end of the ruptured protein is bound up as a thiazolodine residue, which, in some cases, is advantageous in indentification, but generally becomes problematic when it comes to sequence analysis. This problem has been very neatly solved by desulfurization of the N-terminal end with the W-6 Raney nickel leading to a N-terminal alanine unit in place of the original cystine residue.

The attractiveness of this procedure has lead has lead to the development of a variety of positive cyanogen species as shown in CHART-VIII-B. These include 4-nitro-3-carboxy phenyl thiocyanate and the N-cyano compound arising from 4-dimethyl amino pyridine.

←CYSTEINE

Electrophilic functional groups, when attached to cysteine sulfur, give rise to systems that are sufficiently active to

CHART - VII-B

ASP → PRO

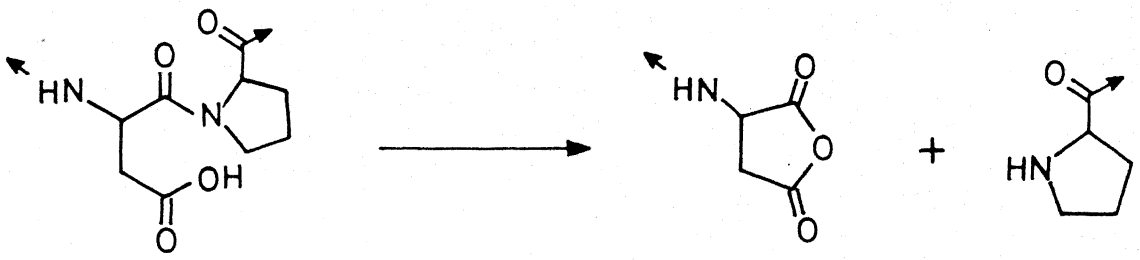
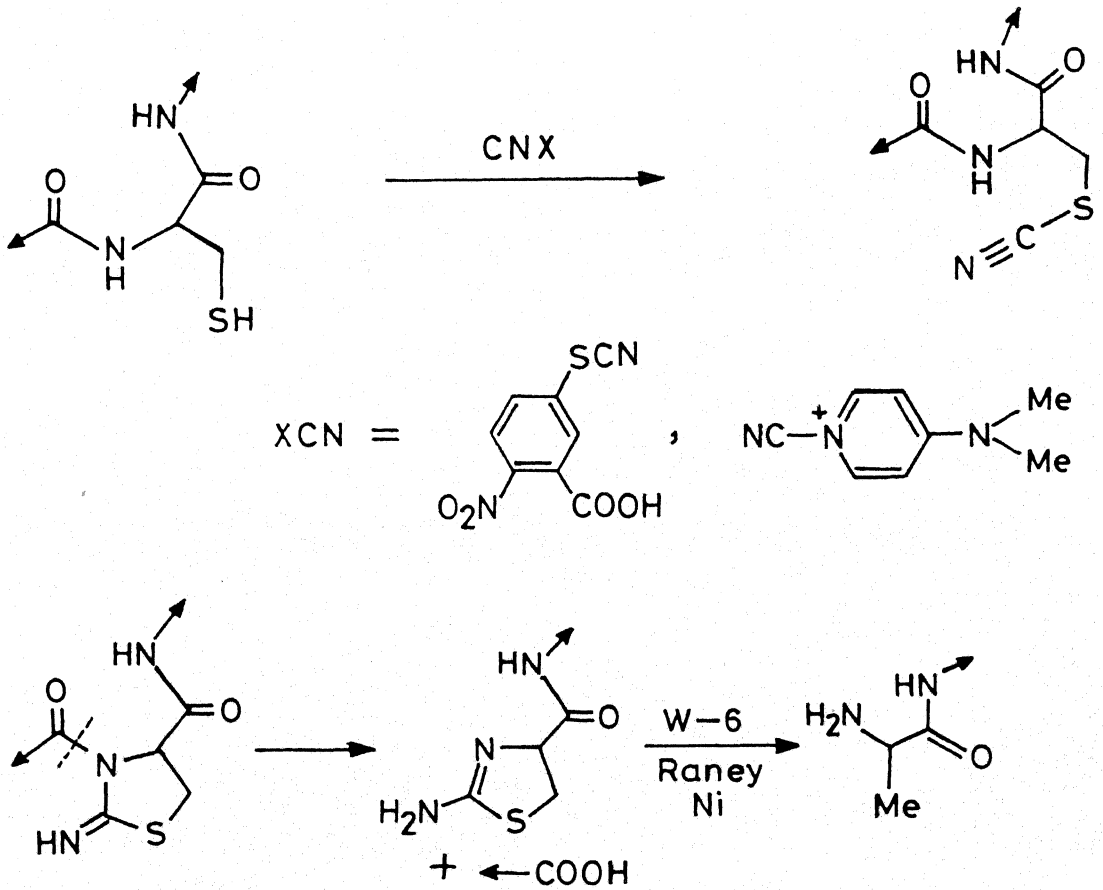


CHART - VIII-B

← CYS



effect nucleophilic addition from the N-H of the peptide residue towards the carboxyl end. The cyclic systems thus formed undergo hydrolysis leading to the rupture of the peptide bond. This has illustrated with the thiocyanate arising from cysteine (CHART-VIII-B). Such intermediates arising from the reaction of cysteine with chloroformates can also lead to peptide rupture (CHART-IX-B[13,14]).

←CYSTEINE/SERINE

The transformation of appropriate side chains to the corresponding dehydro amino acids offers another possibility for protein rupture. Dehydro amino acids can be formed from cysteine and serine as shown in CHART-X-B. In either case, the OH or the SH group has to be made more electronegative by attachment of appropriate ligands. The dehydro amino acid intermediates, possessing an enamine structural unit, undergo hydrolysis leading to a C-terminal amide and a N-terminal 1,2-diketo system. Parenthetically the dehydro amino acid residues could be used to cross- link the protein chains or to attach it to a appropriate substrate via nucleophilic addition (CHART-X-B[15]).

←CYSTEINE

The extension of the cyanogen bromide method, so effectively used for the rupture of a peptide bond proximate to a methionine residue (vide infra) has been extended to cysteine as well. Nor-

CHART - IX - B

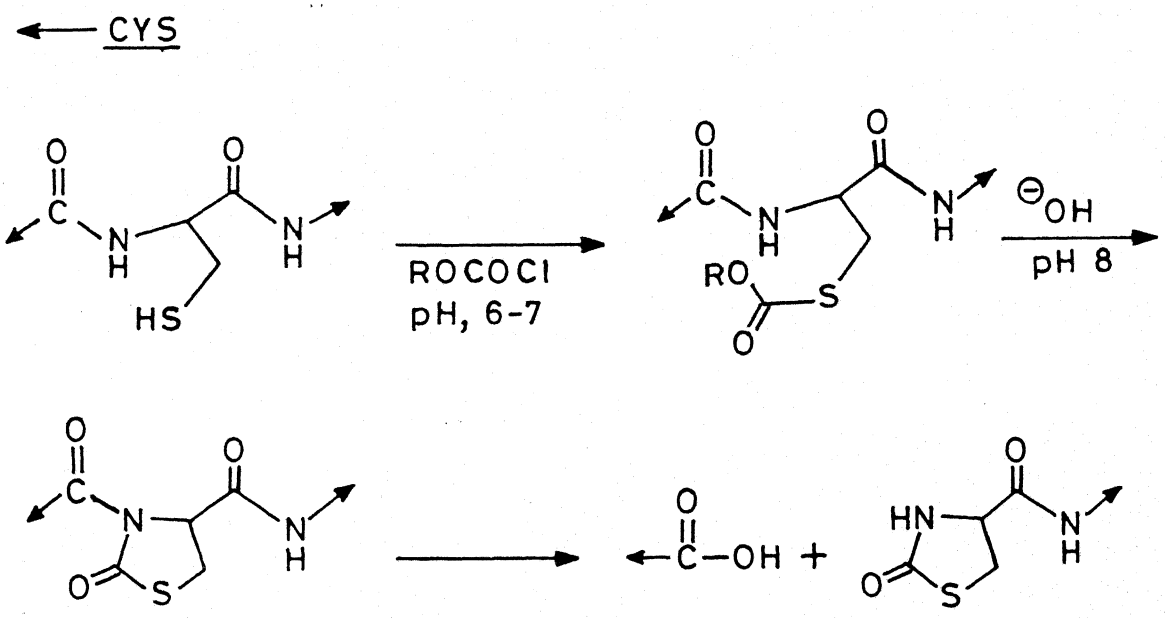
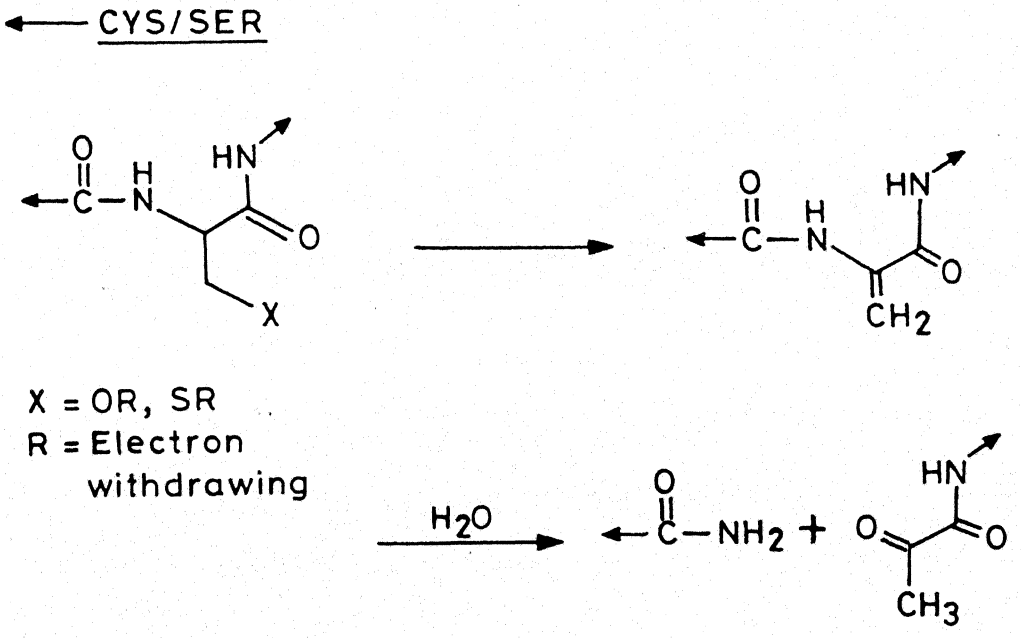


CHART - X - B

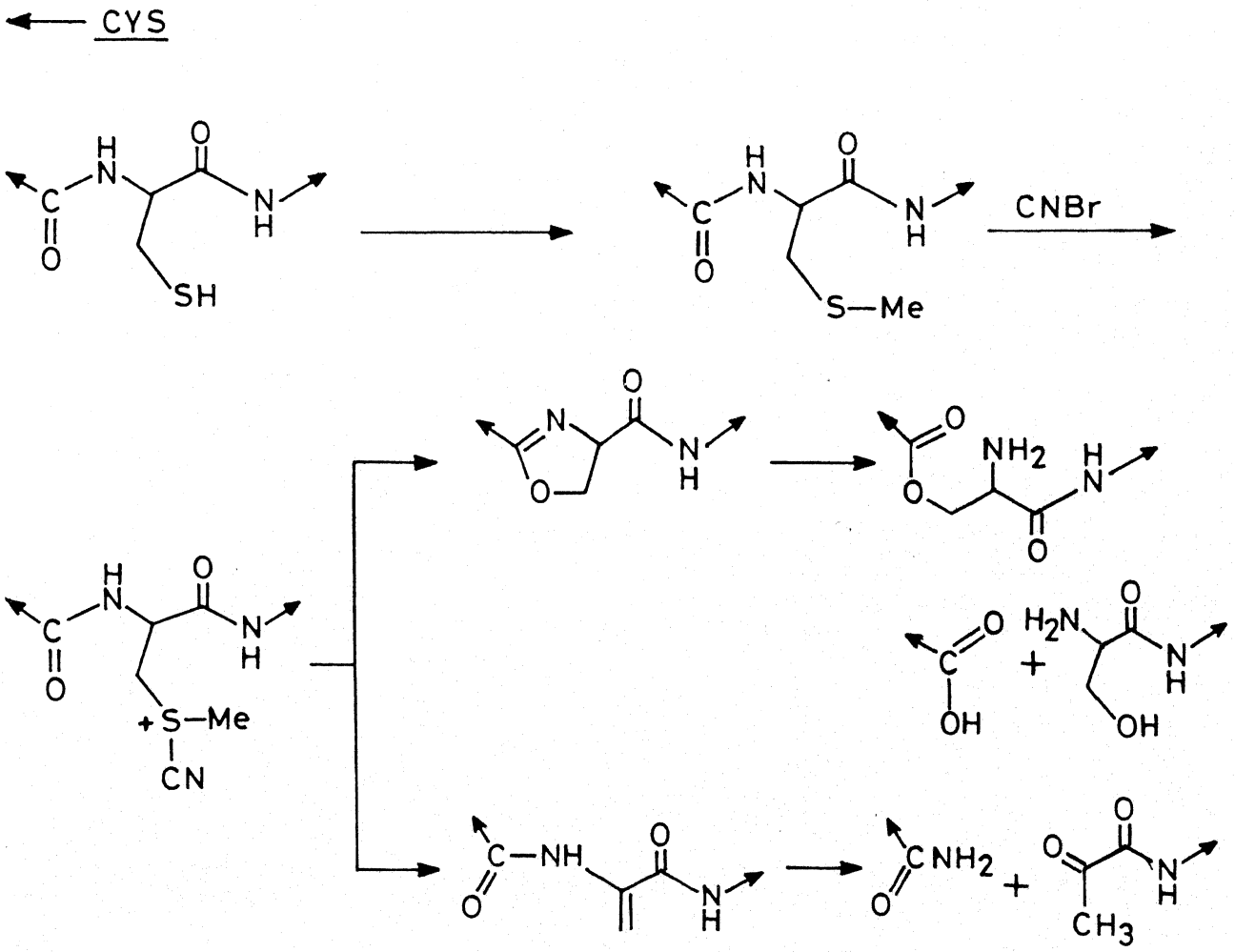


methionine, which can be easily prepared by methylation of cysteine, and which is present in some naturally occurring substances, with CN-Br does not give the β-lactone by pathways similar to that of methionine cleagave where the peptide bond from the carboxyl end can participate in the displacement of the methyl thiocyanate leading to the γ-lactone and eventual rupture (CHART- XI-B). On the other hand, the intermediate S-cyano, S-methyl cyteine undergoes peptide participation from the amino end to form an N protonated oxazoline intermediate, which readily undergoes hydrolysis, thus effecting the N-→O shift of the peptide from the carboxyl end. The resulting ester bond rapidly undergoes hydrolysis giving rise to fragmented products. At higher temperatures the incursion of the elimination-hydrolysis sequence, encountered in the case of Cys/Ser (CHART X.B) becomes important. This method has found use in the rupture of a variety of cysteine containing peptides as well as those which have already S-alkylated cysteine residues (CHART-XI-B[16,17]).

HISTIDINE→

-Keto side chains could readily cleave the proximate peptide bond via the hydrazone. Consequently any of the coded amino acid side chains which could be transformed to such a unit, would provide a methodology for specific cleavage. This unit can be generated from tryptophan, threonine and serine as well as histidine. The generation of the γ-keto unit from histidine side chain proceeds via an interesting mechanistic pathway which

CHART-XI-B

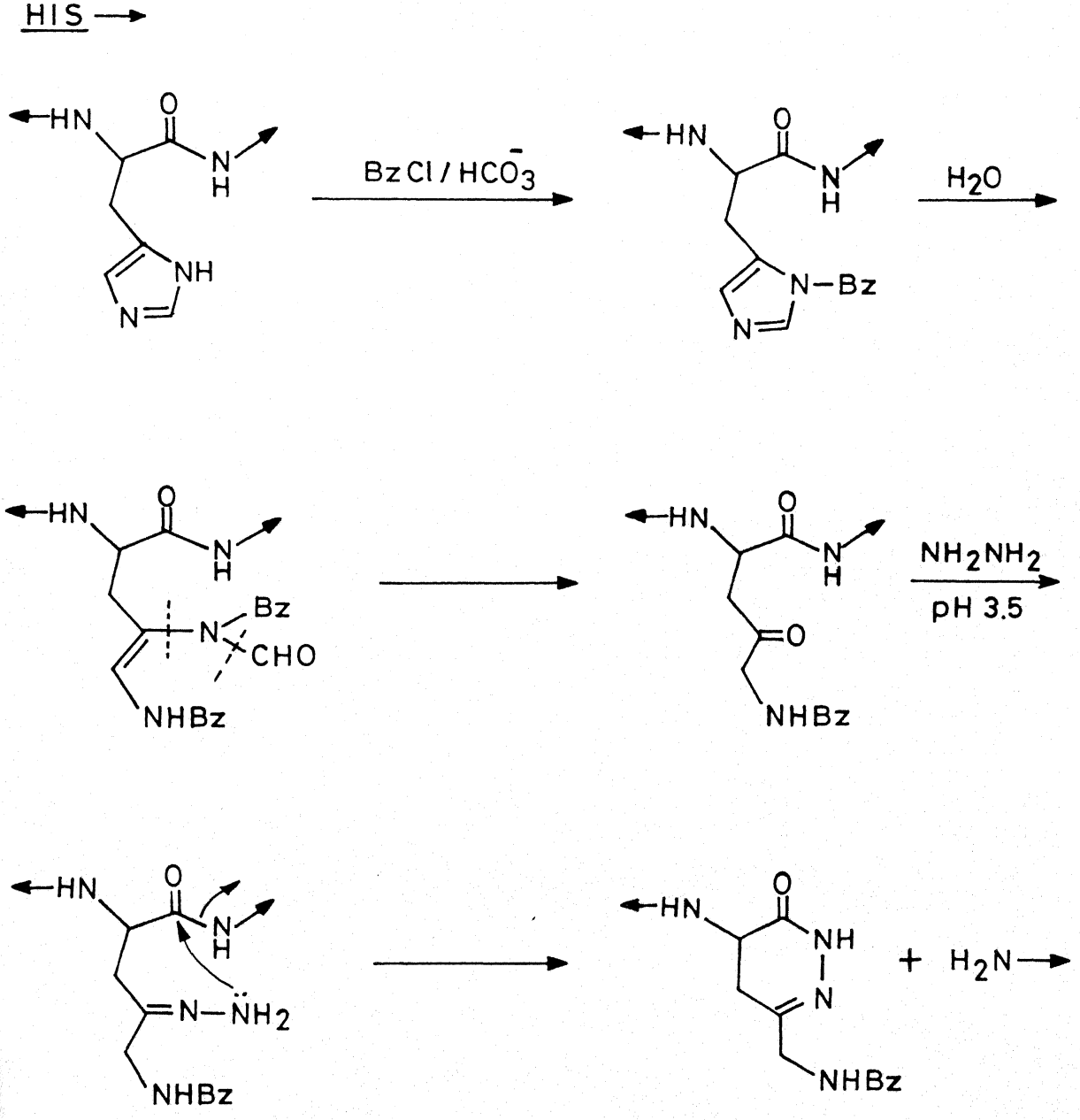


was discovered during studies on protection of the α-amino moiety of the amino acid histidine. Unless the conditions are rigorously controlled, protection by any reagent such as benzoyl chloride or benzyloxy carbonyl chloride in bicarbonate leads to cleavage of the imidazole, ultimatly giving rise to a γ-keto system. The mechanism of this transformation is presented in (CHART-XII-B[13,18]) which involves the expected benzoylation involving both the nitrogens of the imidazole ring, rupture at 2-position and hydrolysis of the enamino system thus created. In the event, it has been found that the γ-keto compound bearing a certain amount of resemblence to N-formyl kynurenine that could be readily obtained from cleavage of the tryptophan side chain, on treatment with hydrazine, under buffered conditions at pH 3.5, leads to the rupture of the proximate peptide bond (CHART-XII-B).

HOMOSERINE→

A rather unusual peptide cleavage was discovered accidentally during the synthesis of a noncoded amino acid canvaline, a close analog of ornithine. Thus, it was found that the homoserine side chain could rupture the proximate peptide bond by brief treatment with 25% TFA in dichloromethane at 0°C (CHART-XIII-B[19]).

CHART-XII-B


HIS
HN
O
N
H
NH
N
BzCl / HCO3
H2O
N–Bz
Bz
CHO
NHBz
NH2NH2
pH 3.5
=O
N–NH2
+ H2N

METHIONINE→

The selective cleavage of the protein backbone taking advantage of a methionine reasidue has perhaps played the most pivotal role, not only with reference to our understanding of the complex structure of proteins, but also in critical situations where such a breakage is required as in the case of humulin synthesis. In addition, methionine being an initiator for protein synthesis, rupture at this site makes it fortutiously a very important operation. The methionine-mediated breakage of the peptide bond from the carboxyl end involves treatment with cyanogen bromide, commonly in aq. 70% formic acid. The methodology by which this rupture takes place is presented in CHART- XIV-B[1a]. The acceptance of elements of CN^+ by the methionine sulfur leads to a highly electrophilic sulfur centre resulting in the rupture of γ-carbon-sulfur bond of methionine by the proximate peptide bond . The resulting γ-lactone undergoes hydrolysis under conditions of rupture leading to a serine residue. In sum, in this method, the methionine residue breaks the peptide bond succeeding it and itself is eventually transformed to homoserine. The procedure has found very important and very spectacular uses. For example, this was useful in the rupture of the 1021 residue of β-galactosidase and, as mentioned earlier, in the rupture of the insulin chains from the β-galactosidase promotor in cloning experiments. Yet another advantage is that the by-products of the reaction are highly volatite and therefore the work-up becomes quite easy. Of

CHART - XIII - B

H. SER →

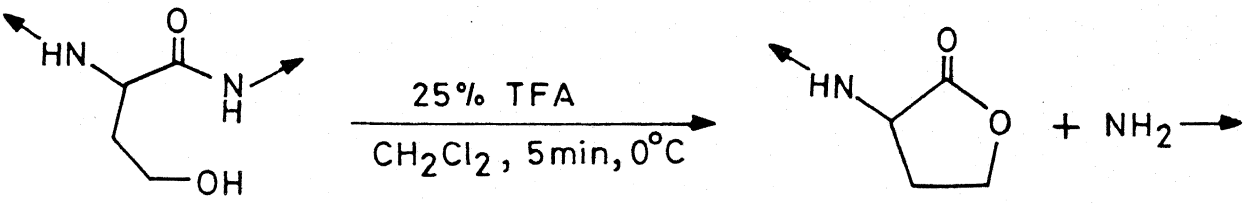
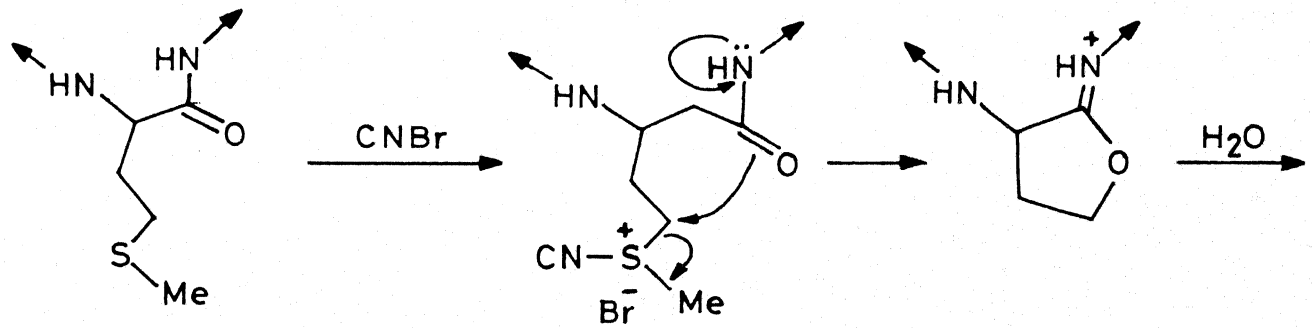
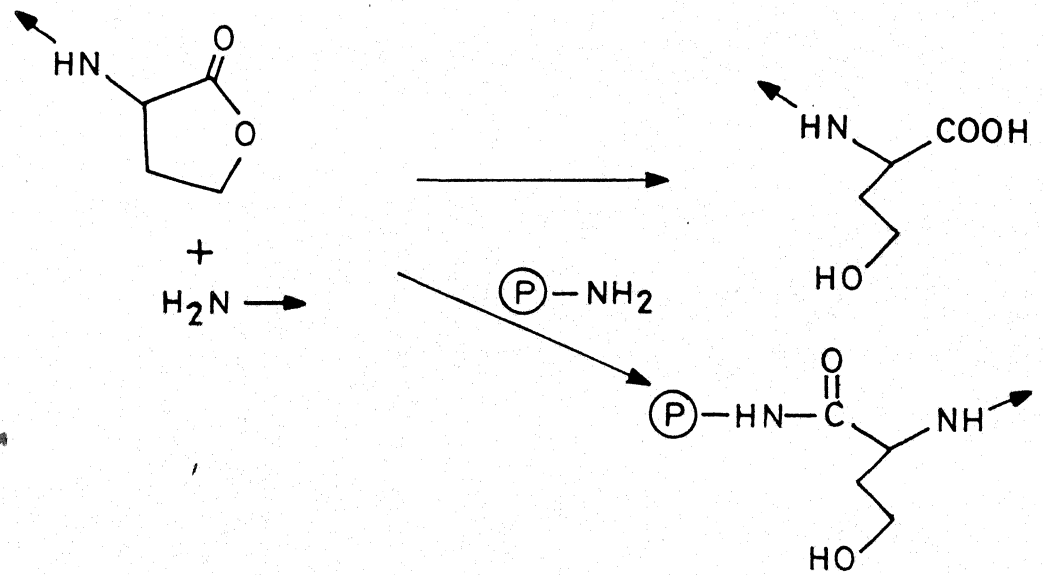


CHART - XIV - B

MET →





particular importance is that homoserine lactone involved after cleavage (CHART-XIV-B) has been shown to readily condense with amino functions attached to a polymer support, leading to immobilization of specific protein fragments on a support (CHART-XIV-B). It could be seen from CHART XIV.B that such an immobilization leads to a serine unit being attached as the first residue.

The cyanogen bromide reaction has also some disadvantages. The obvious one is that tyrosine as well as tryptophan are susceptible to attack by cyanogen bromide, leading to complications. In few cases, the peptide bond is cleaved from the amino end involving a six membered transition state. Fortunately, this pathway is of minor importance. The normal and side reactions associated with the initially formed electrophilic methionine side chain are shown in CHART-XV-B.

Complications also arise if either a serine or a threonine side chain follows methionine. The complication is mechanistically very interesting and is illustrated in CHART-XVI-B[20]. In those cases where the amino acid residue towards the carboxyl end is either serine or threonine, the initially formed reactive intermediates arising from the displacment of the sulfur from the methionine side chain via peptide bond participation, can suffer an intramolecular nucleophilic attack by the proximate hydroxyl function leading to a spiro intermediate. The spiro intermediate, in turn, could lead to, either lack of rupture resulting in the mere transformation of the methionine

CHART - XV-B

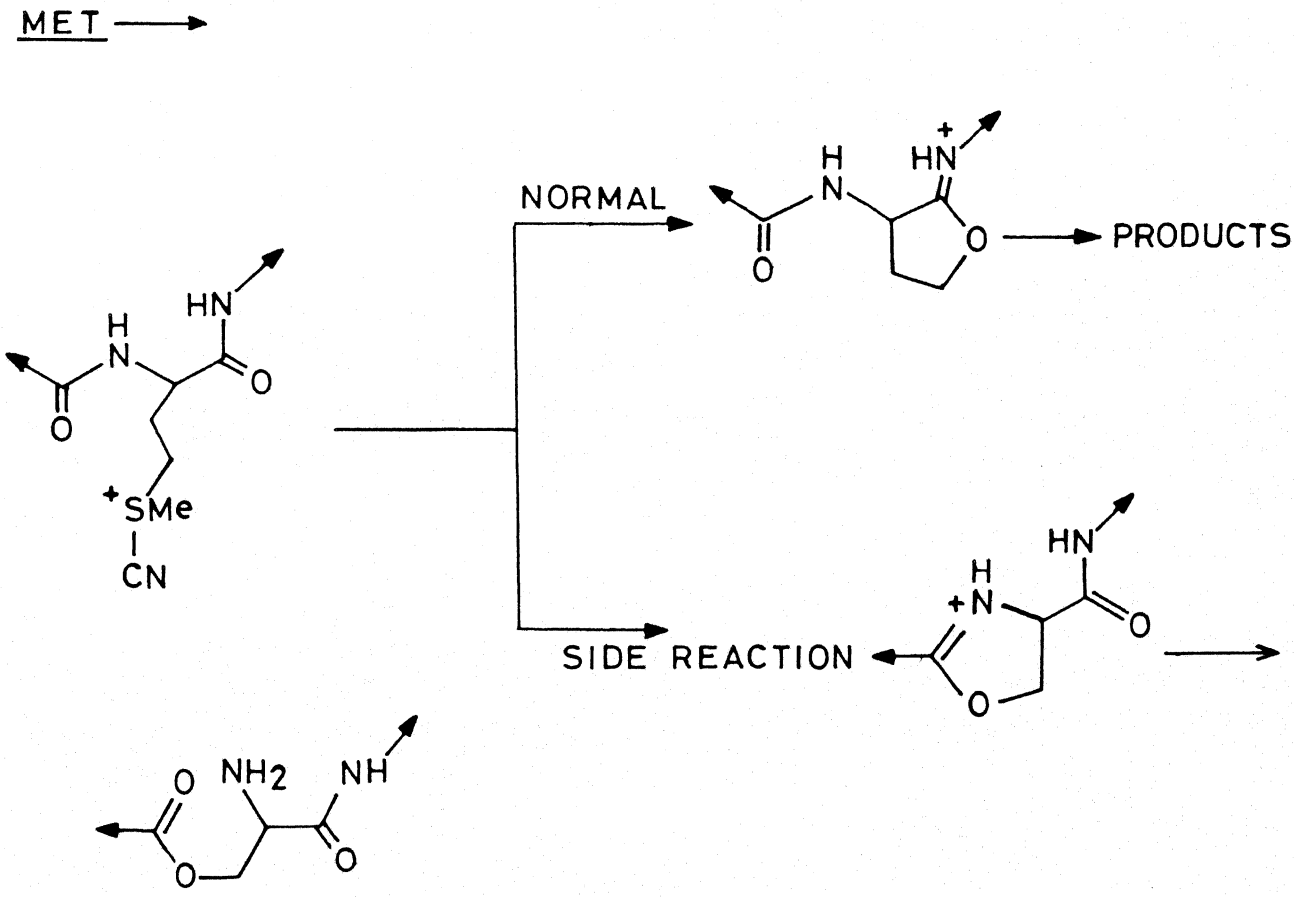
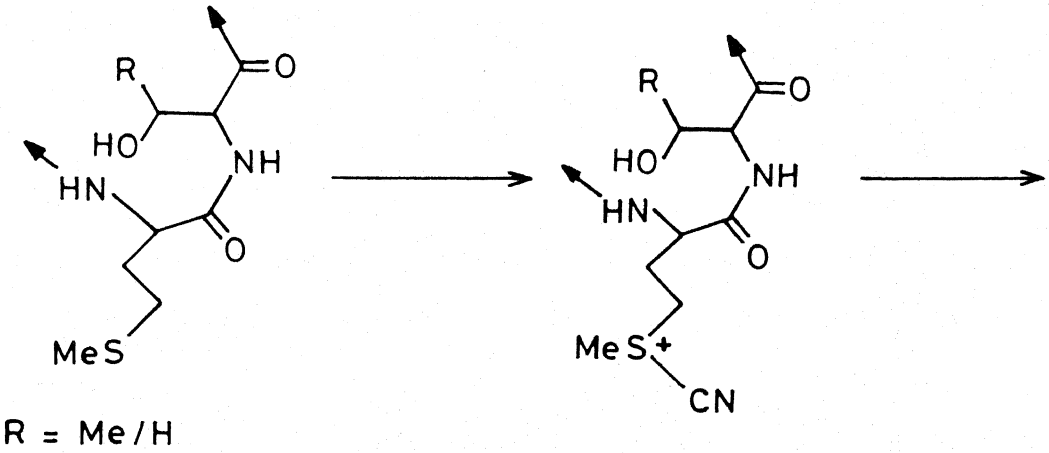
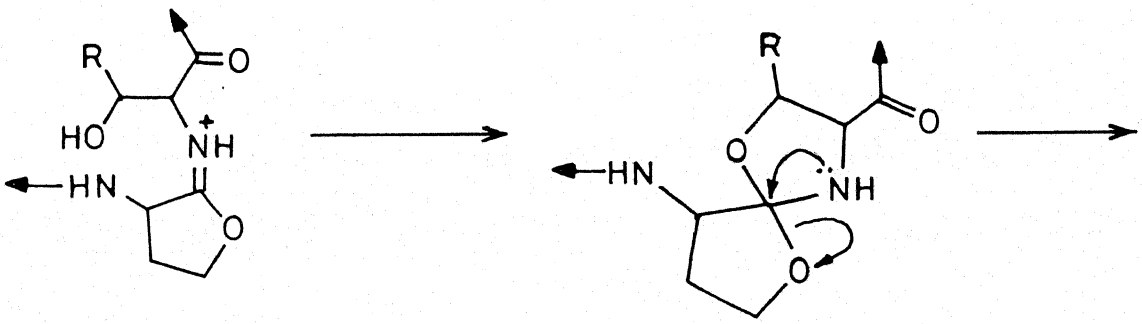


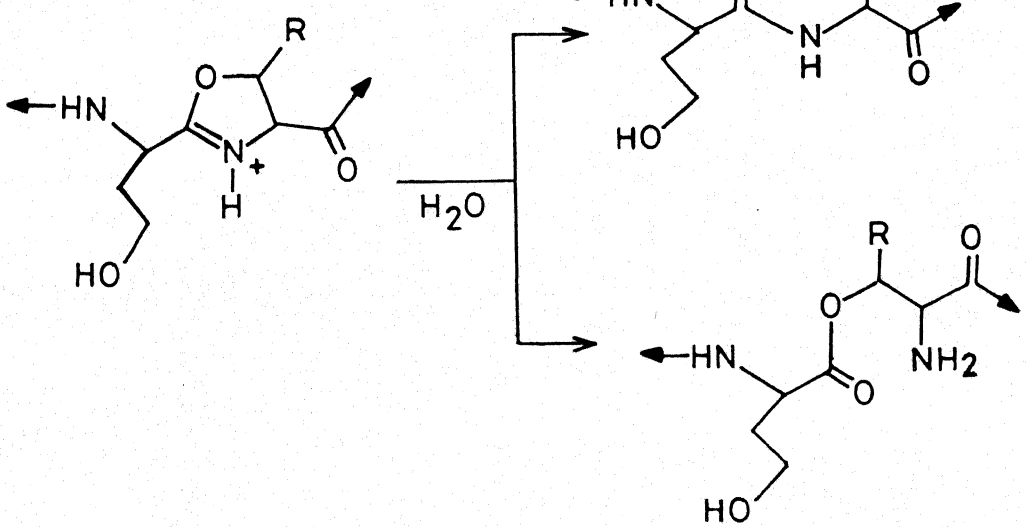
CHART - XVI - B

MET ⟶



R = Me / H





side chain to that of a homoserine, or could lead to, via a depsipeptide intermediate (CHART - XVI-B), the anticipated rupture. Fortunately, by carrying out the cyanogen bromide reactions in those proteins which have a proximate serine/threonine, this complication can be minimised by using trifluoroacetic acid. It could be seen from CHART-XVI-B that any reagent which would make the methionine sulfur a good leaving group can bring about the cleavage. A number of such reagents have been developed (CHART-XVII-B).

METHIONINE→

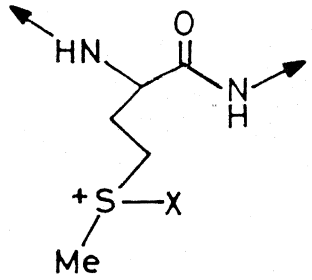
The cleavage of the peptide bond adjacent to that of methionine side chain is normally accomplished by making the sulfur electrophilic. Further, the C-terminal end of such cleaved proteins have homoserine side chain instead of the original methionine. An exception to this is the cleavage brought about in anhydrous hydrofluoric acid at room temperature (CHART- XVIII-B[21]). In this case, the sulfur lone pair initiates the peptide rupture, leading to cleavage with retention of the methionine side chain at the C-terminal end of the cleaved fragment.

PHENYL ALANINE→

Even the traditionaly unreactive benzene ring in the phenyl alanine side chain can be modified to effect the rupture at the

CHART - XVII-B

MET →



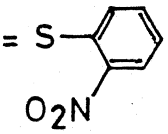
X = R O
X = —CH_2CNH_2
X = SCOOMe
X = S– 

CHART - XVIII-B

MET →

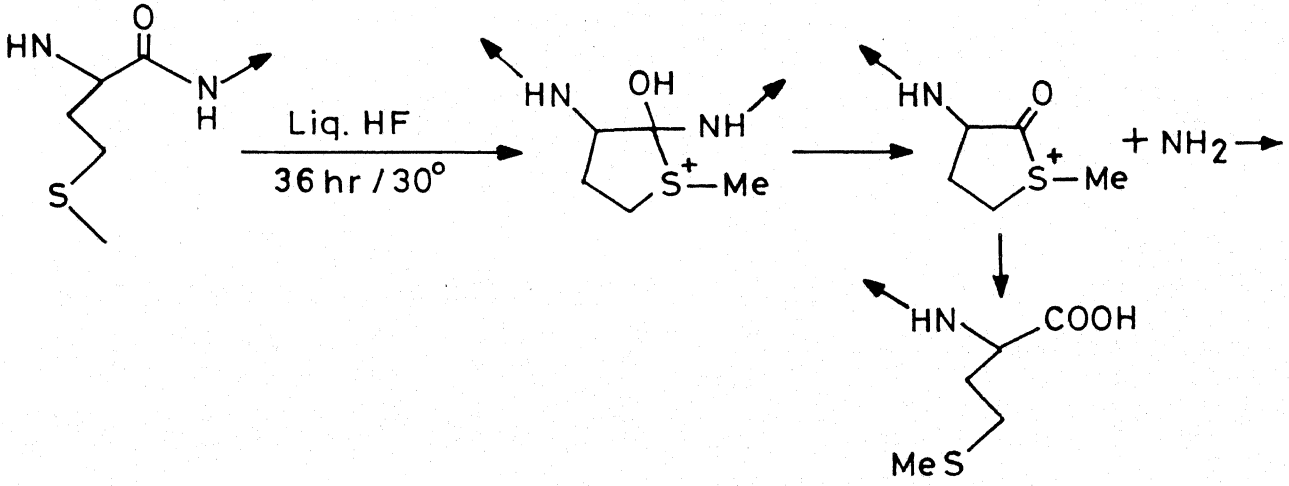
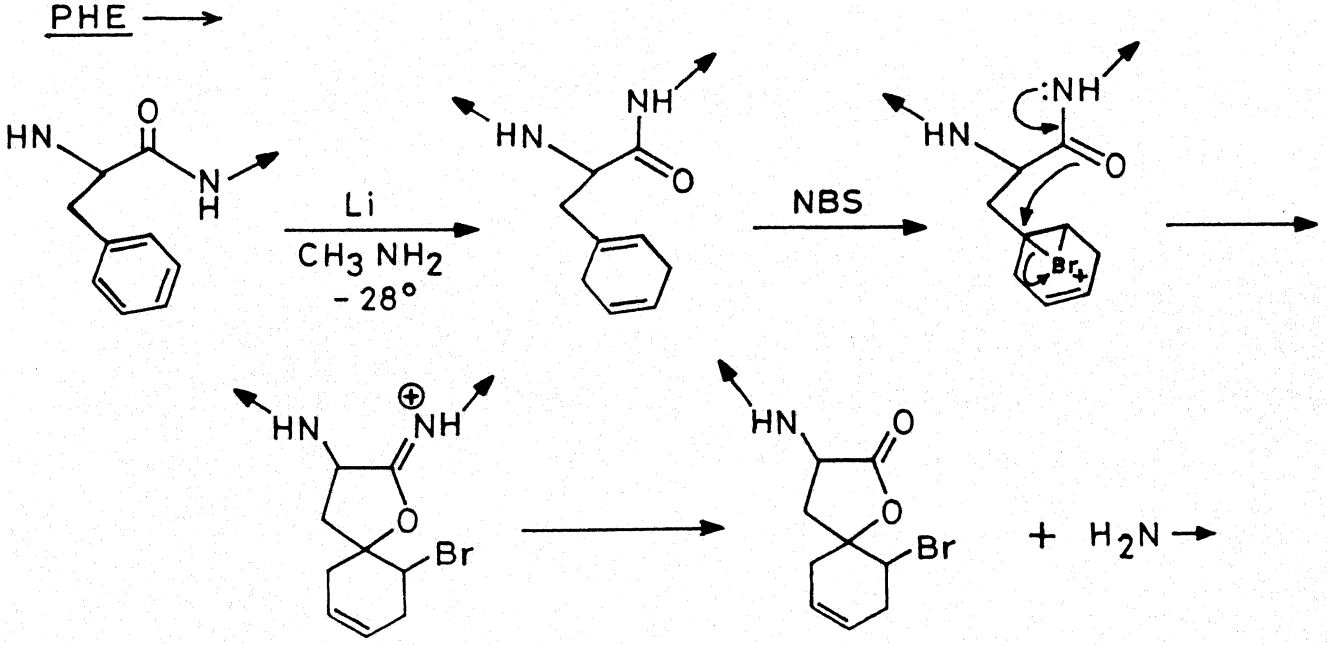


CHART - XIX-B

PHE →



proximate peptide site. The methodology envisaged is simmilar to that in the case of tyrosine. However, with an unactivated benzene ring, it has to be transformed to a π bonded system prior the treatment with NBS. This has been achieved by reduction in Li/CH_3NH_2 at - 28 ^{o} C. The resulting 1, 4 diene on treatment with NBS, followed by hydrolysis leads to the rupture of the peptide bond (CHART-XIX-B[22]). The method, because of the extreme conditions involved, is obviously of rather limited advantage, but could find use in circumstances where the peptide chain contains largely hydrophobic residues such as in signal sequences.

←PROLINE

The peptide bond arising from proline is quite different from others because of the tertiary nitrogen. Further, the cyclic nature of the proline residue makes the π bond order of this peptide bond lower, thus enhancing the C-N reactivity. This aspect is taken advantage of in the selective cleavage of proline residues from the amino end by electron doners such as sodium/liquid ammonia, sodium hydrazide/ anhydrous hydrazine/ ether and LAH/THF. These reduce the C-N sigma bond leading to an aldehyde at one end and a proline at the other (CHART- XX-B[23]). Invariably, the C-terminal aldehyde is further reduced to the corresponding alcohol. The reaction has found rather limited use, although sodium hydrazide in hydrazine/ether is a very superior reagent and brings about the cleavage even at room temperature.

SERINE/THREONINE→

An interesting method for the rupture of the bond succeeding either serine or threonine involves prior oxidation of the hydroxyl group to the carbonyl using DMSO-DCC in presence of phosphoric acid. The oxidized side chains thus obtained can be cleaved, either with hydroxylamine leading to rupture of the peptide bond with concomittant formation of an isoxazole residue, or with phenyl hydrazine leading to a pyrazole, both at the carboxyl terminal. The method is useful for the cleavage of small peptides. (CHART-XXI-B[24,25]).

SERINE/THREONINE→

The chloroformate intermediate arising from serine or threonine, on refluxing with xylene, leads to highly reactive cyclic lactones via intramolecular cyclizations initiated by the nitrogen of the proximate peptide bond. These, on treatment with dilute alkali, yield cleaved peptide (CHART-XXII-B[26,27]). This method has found limited application since the initial acylation and subsequent cyclization has to be carried out in non polar solvents in which few peptides are soluble.

←SERINE/THREONINE

The coded amino acids serine and threonine possessing, respectively, a primary and a secondary hydroxyl group, can bring

CHART-XX-B

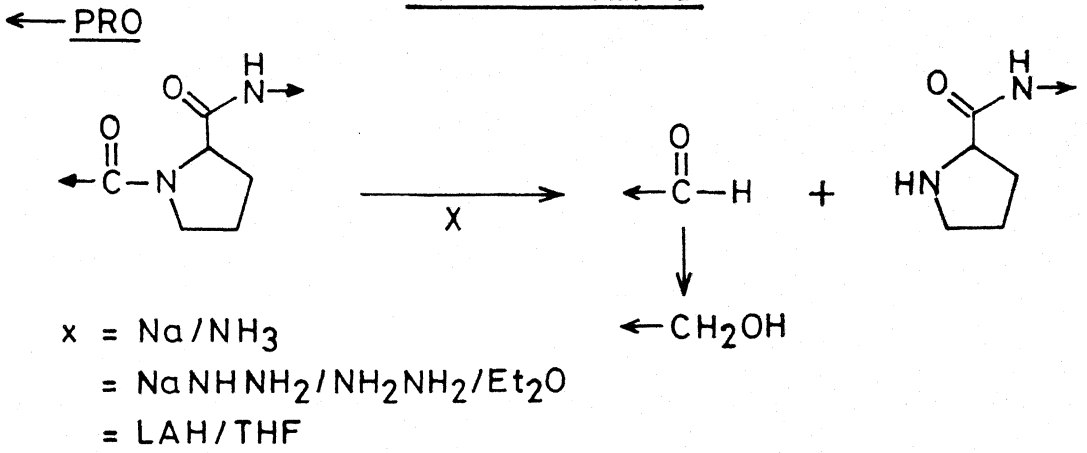
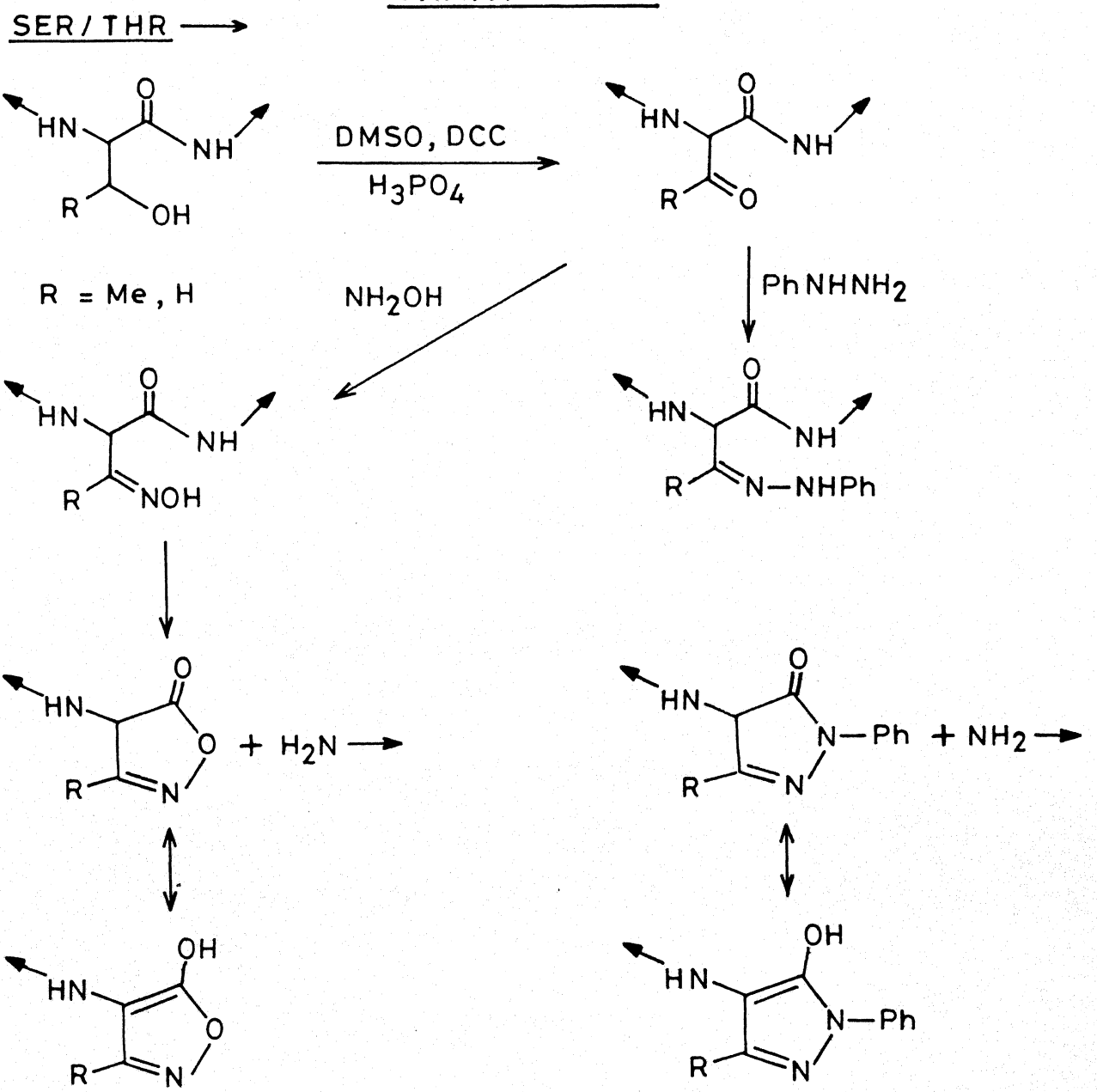


CHART-XXI-B



about the cleavage of the peptide bond at the amino end using strong acids like sulfuric, formic and hydrofluoric, under anhydrous conditions. The mechanism by which this rupture takes place is presented in (CHART- XXIII-B[28]). It involves the interaction of the side chain with a protonated proximate amide function, loss of water and readdition, thus shifting the peptide from a nitrogen to an oxygen function. The resulting depsipeptide is quite easily hydrolyzed. The requirement of very strong acids necessarily makes this procedure somewhat unspecific and in some cases the intermediate depsipeptide is transformed back into the original protein via reversible intramolecular reactions. Hence this method has not played a very important role in structural elucidation of proteins.

TRYPTOPHAN→

The enormous potential arising from deployment of chemical tools towards protein rupture is dramatically illustrated with tryptophan side chain which could bring about the breakage of the adjacent peptide bond with N-bromosuccinimide. In view of the complexities of this reaction, it must be termed as one of the true discoveries, because the nature of the pathways makes any prediction impossible (CHART -XXIV-B[1a]). The process is initiated with Br^+ equivalent, which, as expected, is picked up at the 3-position of the indole. The reactive intermediate arising from this undergoes hydration, loss of elements of HBr and protropic shift leading to an oxindole which, possessing a very highly reactive C-H bond, is readily brominated with NBS.

CHART-XXII-B

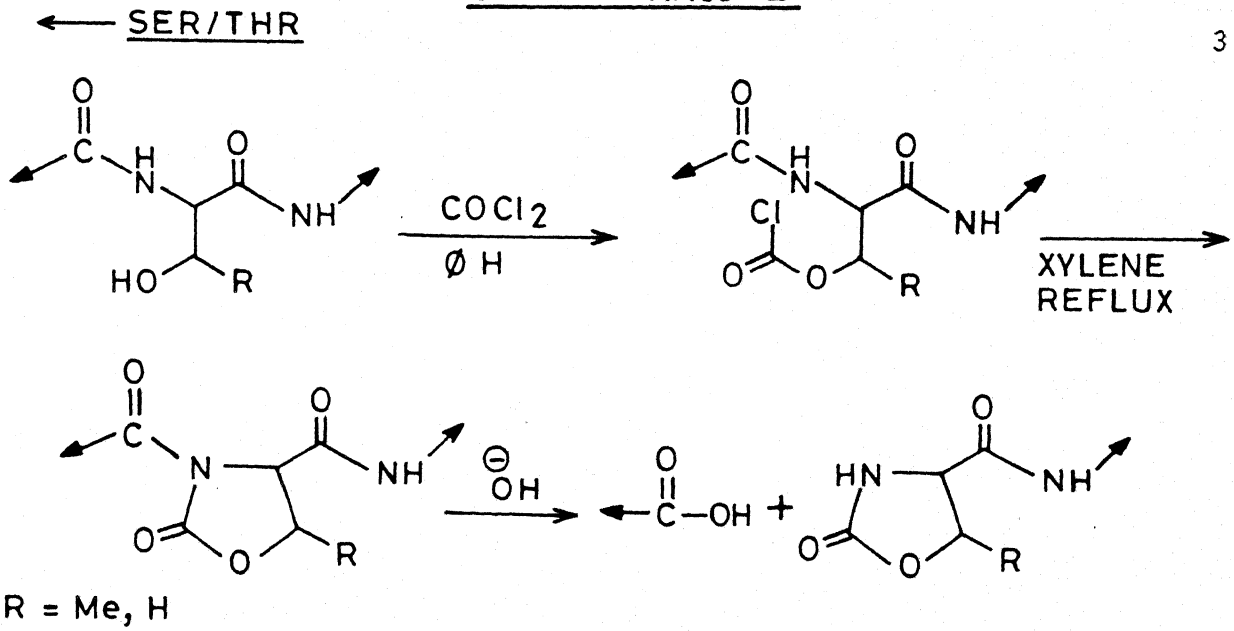


CHART-XXIII-B

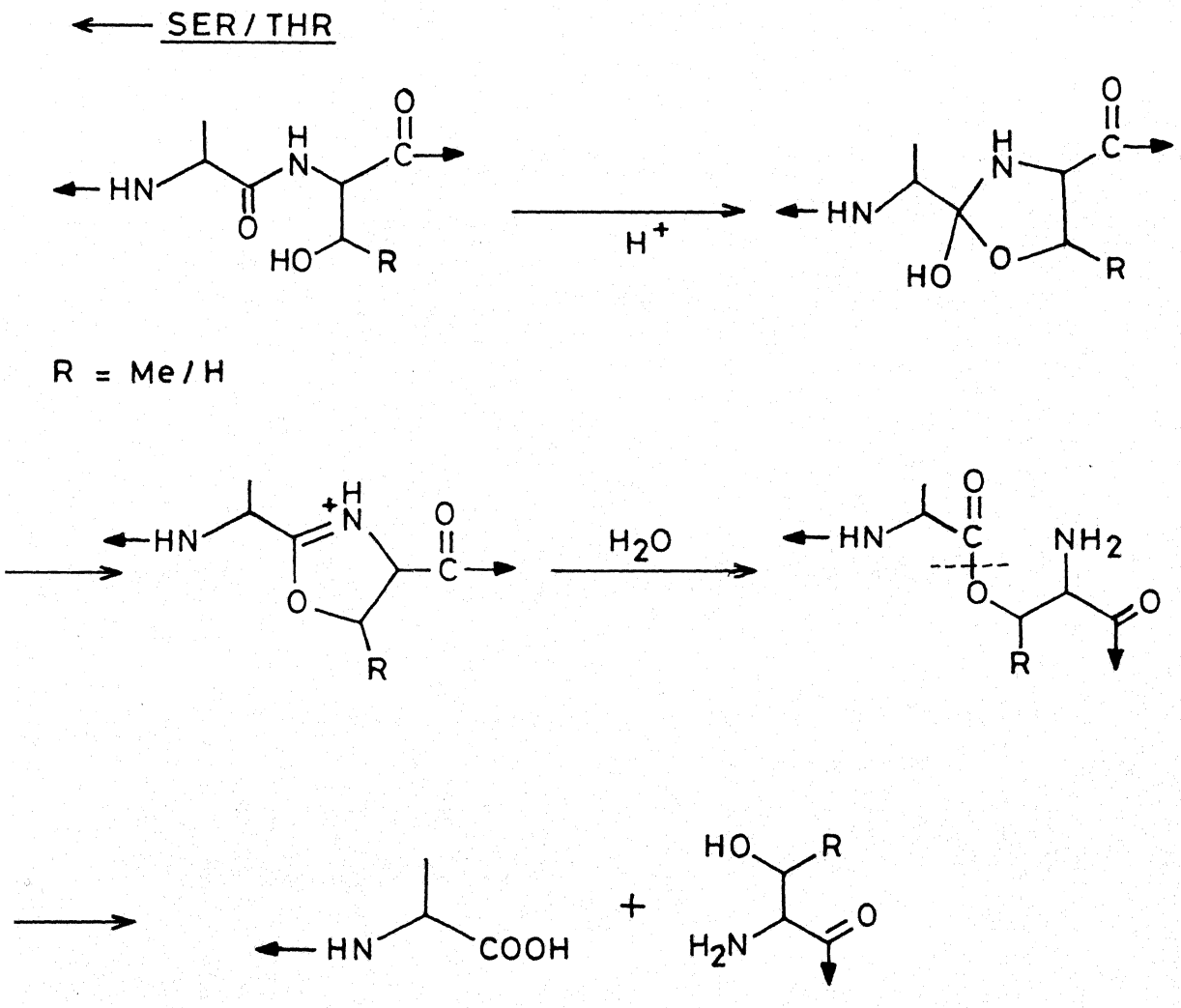
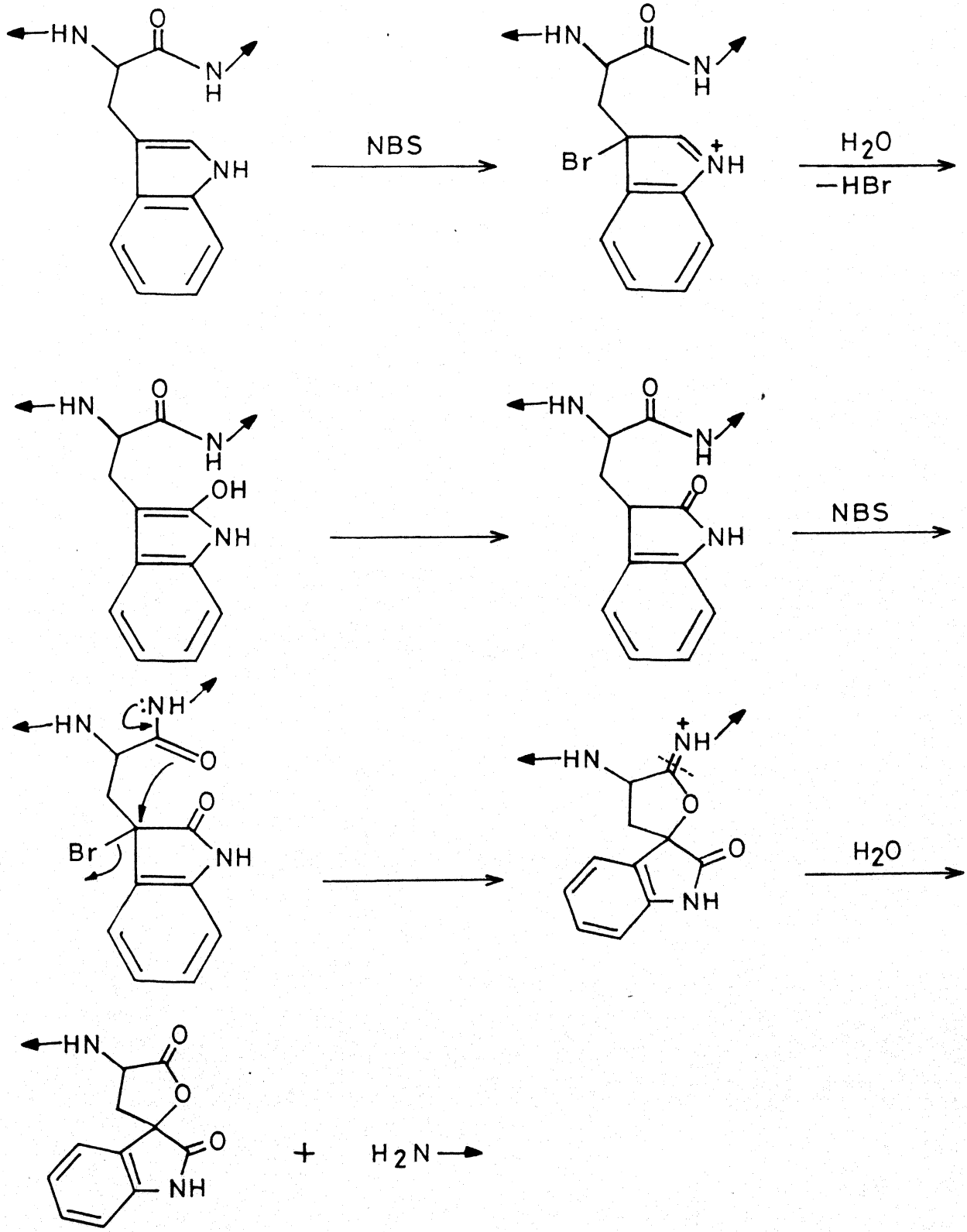


CHART - XXIV - B

TRP →



The resulting highly labile tertiary bromide undergoes displacement by participation of the proximate peptide bond leading to a spiro intermediate, which, via hydrolysis leads to ring rupture. The entire sequence of reactions is attended with sufficient mechanistic complexities with the result that not only this procedure is very non-selective, but also the yields are very low. In addition, other coded amino acids side chains such as tyrosine and histidine interfere.

In some cases the peptide bond from amino side could also undergo cleavage. When the tryptophan residue is at the N-terminal end, the cleavage does not take place. At least some of the complexities associated with the use of NBS is because of its high activity leading to decreased selectivity. One of the most promising brominating agents in which the positive bromine activity is moderated is called BNPS skatole (CHART- XXV-B[29]). The high selectivity of BNPS skatole, which brings about the cleavage of the succeeding peptide bond with reference to the tryptophan in 75% aq. acetic acid, is reflected by the fact that the only amino acid side chain that interferes is methionine.

The finding that a positive halogen can bring about the rupture of a peptide bond adjacent to a tryptophan residue has led to the development of various reagents that would achieve this purpose. These include DMSO/HCl,DMSO/HBr,o-iodosobenzoic acid/HCl and N-chlorosuccinimide (CHART-XXV-B[30]). Tryptophan is one of the rarest amino acids present in proteins. Therefore,

cleavage adjacent to this residue is very attractive in the sense that even most complex proteins can be broken down into few fragments.

Cleavage of peptide bonds adjacent to the tryptophan residue can be achieved via ozonization to N-formyl kynurenine which, interestingly, in the bicarbonate buffer for 4 hr leads to the cleavage of the peptide bond. Yet another rare procedure for bringing about the rupture of the peptide bond adjacent to the tryptophan residue is to react the kynurenine with hydrazine/acetic acid at pH 3.5, leading to the rupture via the intermediate hydrazone (CHART-XXVI-B[31]). This method of cleaving the tryptophan via kynurenine has not been found to be very attractive not only because the yields are very low, but also residues such as methionine, cysteine and tyrosine are affected. The recently introduced reagent 4-^{t}-butyl iodoxybenzene cleanly ruptures tryptophan to formyl kynurenine and perhaps with this modification, the kynurenine intermediated method could be further examined.

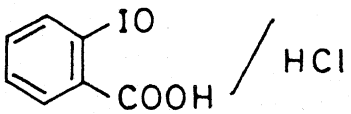
TYROSINE→

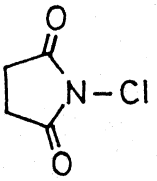
The reactivity of tyrosine as a highly electron rich substrate has been referred to earlier. The tyrosine residue can bring about the rupture of a proximate peptide bond via a mechanistic pathway not too dissimilar to that of tryptophan (CHART-XXVII-B[32]). The reaction proceeds via per bromination of the electron rich p-hydroxy aromatic ring present in tyrosine to

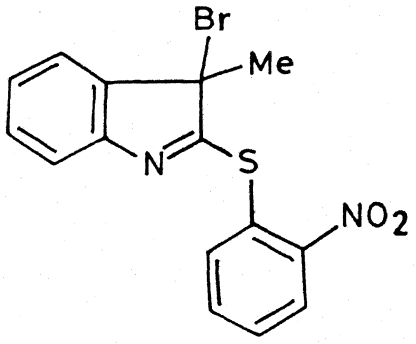
CHART - XXV - B

TRP →

DMSO / HCl
DMSO / HBr



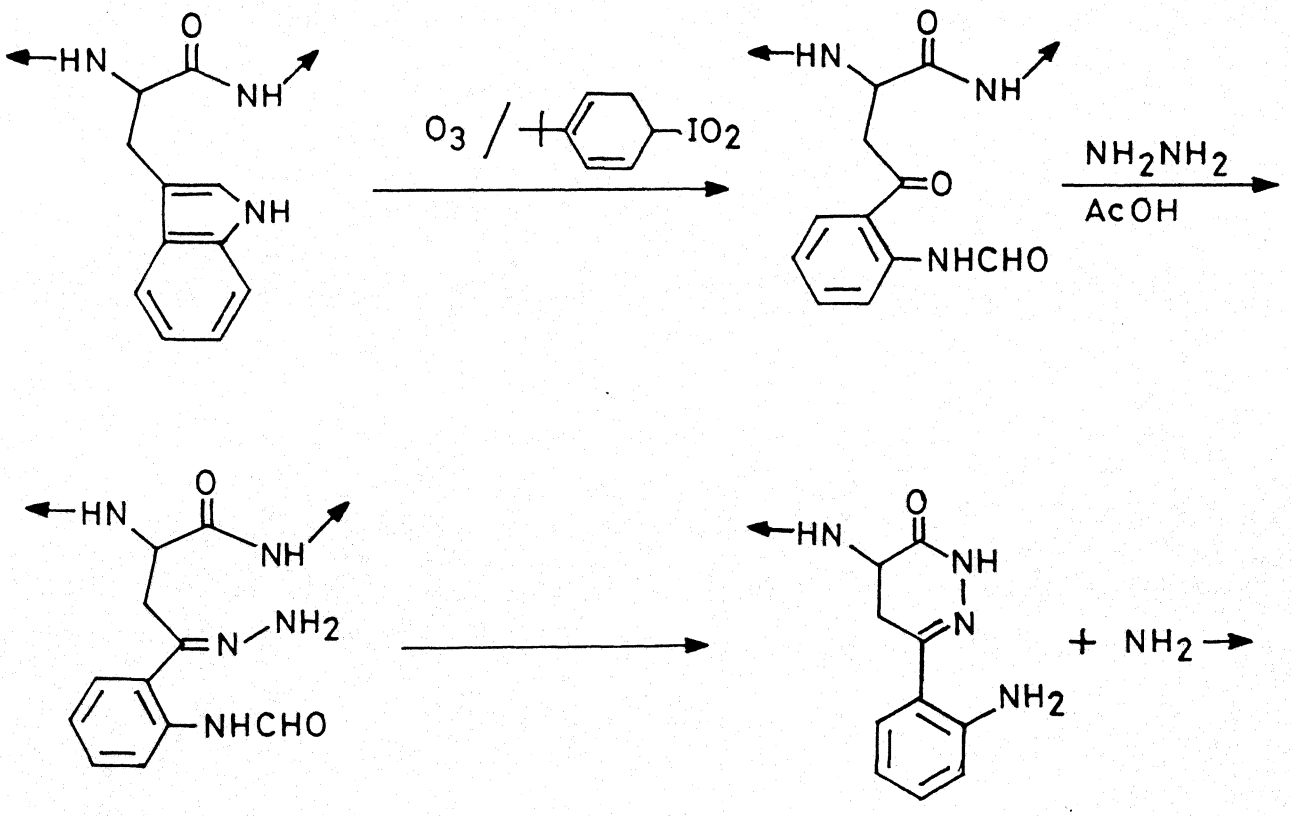




BNPS SKATOLE

CHART - XXVI - B

TRP →



a tribromoquinone possessing a tertiary reactive bromide very similar to that encountered in the cleavage of tryptophan (CHART-XXVI-B)

Intramolecular displacement of this tertiary bromide via the proximate peptide bond followed by hydrolysis leads to the rupture of the protein chain. This method has found use in some highly special but remarkable circumstances. Thus, the six tyrosine residues present in S_2- carboxymethyl ribonuclease have been fully taken advantage of to clearly break this very complex molecule. A rather interesting fact is that histones , the highly basic proteins which are present in the nucleus of eucaryotic systems, possess no tryptophan and therefore in these cases the tyrosine residue can be taken advantage to cleave the peptide bond without having to face the complications usually encountered when tryptophan is present, although the reaction of tyrosine with NBS is much faster. Like in the case of tryptophan, the N-terminal tyrosine is not effective (CHART-XXVII-B).

TYROSINE →

A rather innovative method for the cleavage of the peptide bond proximate to a tyrosine side chain is via anodic oxidation wherein the loss of elements of $2\bar{e}$ from the aromatic moiety is compensated by the peptide participation (CHART-XXVIII-B[33]).

CHART - XXVII - B

TYR →

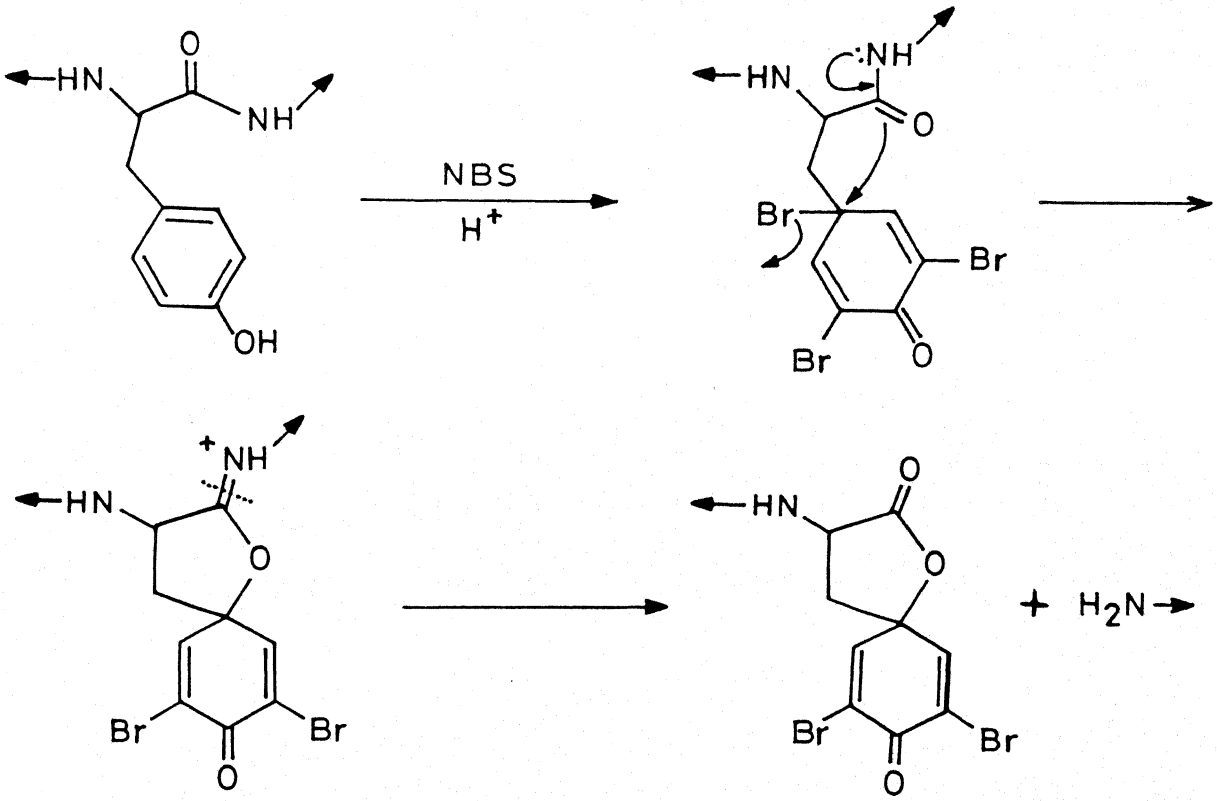
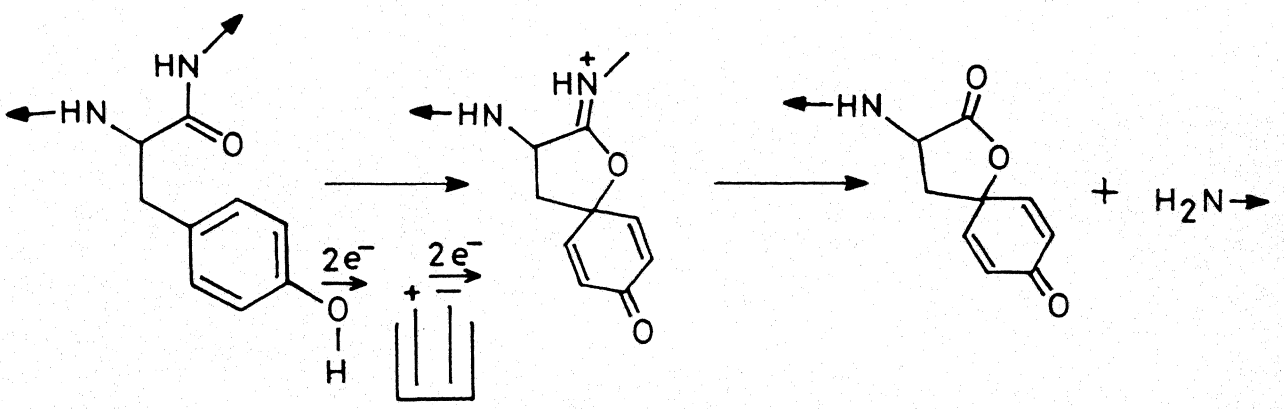


CHART - XXVIII - B

TYR →



SECTION C. PRESEET WORK

General Introduction:

The focus of the present work is directed towards the possible understanding of rationale pertaining to the selection of the 20-coded amino acids amongst the infinite variety possible and from over 500 such structures that exist in nature[34]. It is hoped that such an understanding would not only provide an insight relating to components that make up the functional system, but also would be of help in the synthesis of tailor-made compounds having specified and desirable properties.

Any such analysis should begin with the genetic code, presented in CHART-C-I, a striking feature of which is the non-random degeneracy pertaining to the codes that vary from zero dgeneracy, such as that exist in methionine and tryptophan, to as many as six codons for leucine, serine and arginine. The genetic code must hold clues pertaining to the evolution of the functional system, which is intimately associated with the choice of the coded amino acids.

CHART-C-II illustrates the arrangement of the coded amino acids on the basis of codon degeneracy, noteworthy features of which are that, even presently, seven of the 20 coded amino acids have a defacto doublet code (A), and the five pairs of amino acids which share a common doublet, with the last letter assigned

CHART - C - I

First position (5' end)	Second position				Third position (3' end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Term	Term	A
	Leu	Ser	Term	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

TABLE-C-I

	Gly	Ala	Val	Leu	Ile	Met	Cys	Ser	Thr	Asn	Gln	Asp	Glu	Lys	Arg	His	Phe	Tyr	Trp	Pro
Gly		44	38				16	16				21	24		43				13	
Ala	58		41					35	39			23	26							36
Val	10	37		48	18	12						20	23				16			
Leu	2	10	30		16	21		13			16				22	11	41		9	26
Ile		7	66	25		25		15	17	18							16			
Met	1	3	8	21	6				11					19	7					
Cys	1	3	3		2			24							10		12	15	16	
Ser	45	77	4	3	2	2	12		41	15					30		13	15	8	24
Thr	5	59	19	5	13	3	1	70		17				24	12					28
Asn	16	11	1	4	4			43	17			19		49		13		18		
Gln	3	9	3	8	1	2		5	4	5			23	24	13	26				15
Asp	16	15	2		1			10	6	53	8		49			15		20		
Glu	11	27	4	2	4	1		9	3	9	42	83		30						
Lys	6	6	2	4	4	9		17	20	32	15		10		20					
Arg	1	3	2	2	3	2	1	14	2	2	12	9		48		8			13	20
His	1	2	3	4			1	3	1	23	24	4	2	2	10			13		10
Phe	2	2	1	17	9	2		4	1	1					1	2		16		
Tyr		2	2	2	1		3	2	2	4			1	1		4	26			
Trp				1				2							3		1	1		
Pro	5	35	5	4	1		1	27	7	3	9	1	4	4	7	5	1			

Observed Number of Replacements

Expected Number of Random Mutational Events

to a pyrimidine code for one paratner, and a purine for the other (B). That this special relationship amongst the five pairs is not a fortuitous occurrence, is convincingly brought out in TABLE-C-I, which presents, inter alia, an analysis of the relative frequencies of coded amino acids replacements, observed in a total of 1572 examples of closely related proteins. TABLE-C-I shows that there is an overwhelming preference for mutations that would result in the interchange of these partners over any other. All these, coupled with similarity exhibited amongst most of the pairs, support the notion that they are evolutionarily related in the sense that the original degeneracy, when only one of these partners were present, is lifted to accommodate the other. Methodologies directed at the chemical interchange of these partners would have multifaceted significance and the glutamine-histidine pair has been chosen in the present study.

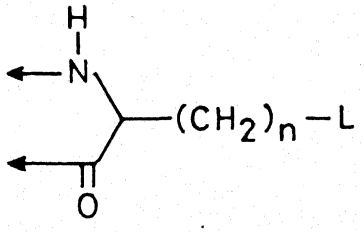
The functional system, made up of the 20 coded amino acids, could be analyzed in terms of three structural elements: a. the generally invariant peptide back bone that carries the chiral centre and contributes to the secondary structure, b. the methylene spacer, which is crucial to the precise structure of the protein and c. the end group, involved in polar and hydrophobic interactions. In the case of proline, which being cyclic possesses no end group, the entire unit contributes to the specific structure of the protein frame work (CHART-C-III).

During translation, the selection of even so closely related pairs such as, asparagine and glutamine , aspartic acid and

CHART-C-II

A	B	D
LEU (CU)	PHE-LEU (UU/U, C-A, G)	ILE (AU/U, C, A)
VAL (GU)	SER-ARG (AG/U, C-A, G)	MET (AUG)
SER (UC)	ASN-LYS (AA/U, C-A, G)	E
PRO (CC)	HIS-GLN (CA/U, C-A, G)	CYS (UG/U, C)
THR (AC)	ASP-GLU (GA/U, C-A, G)	TERM (UGA)
ALA (GC)	C	TRP (UGG)
GLY (GG)	TYR-TERM (UA/U, C-A, G)	

CHART-C-III



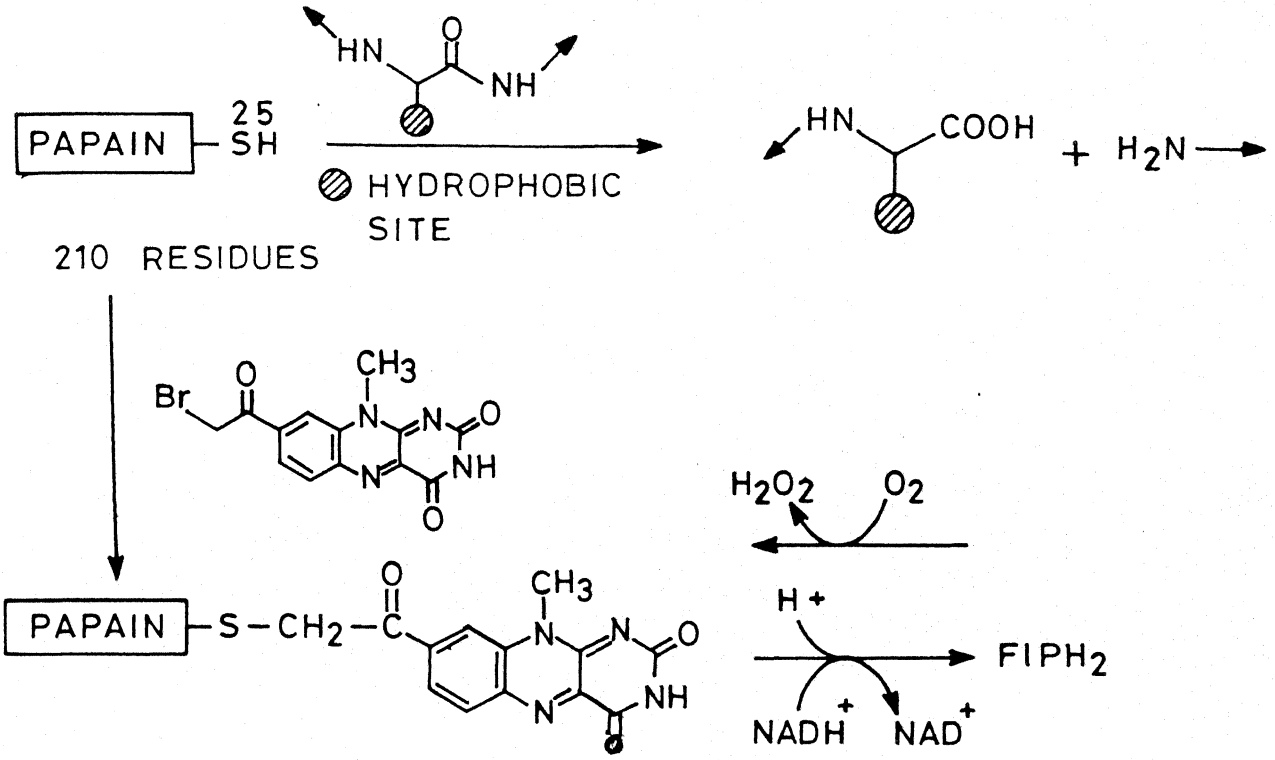
n	Amine acid	
0	Glycine, Valine, Isoleucine, Threonine	4
1	Alanine, Leucine, Phenyl-alanine, Tryptophan, Tyrosin, Histidine, Asparagine, Cysteine, Serine, Aspartic acid	10
2	Methionine, Glutamine, Glutamic acid	13
3	Arginine, Proline	2
4	Lysine	1
		20

glutamic acid, that differ by a mere one methylene group, is made without error. Indeed, the necessity for this has been brought out by the recent work on triose-enol isomerase, wherein, the replacement of glutamic acid with aspartic acid at the active site was found to lead to drastic reduction in the enzymatic activity[35]. Viewed in this context, lysine enjoys a unique position, in the sense that the basic amino function is kept farthest- 4 methylenes away from the peptide backbone. The obvious explanation for keeping the NH_2 function away from the peptide back bone, would be that proteins, having such a residue placed closer, are not viable, either because of the inability of such amino acids to be part of the translational machinary, or due to possible rupture of the protein via intramolecular reactions. This aspect has been examined in the present work.

The observed fact that across the living domain, reflecting millions of years of evolutionary endeavours, the functional systems consist of the same 20 amino acids is truly remarkable. Indeed, the fidelity with which this order is maintained, is best reflected in the fact that in structural proteins like collagen, the inadequacies pertaining to maintenance of proper structures, with a normal compliment of coded amino acids, is overcome via remarkable post translational operations. This is particularly true of proline and lysine residues, which are generally transformed, to respectively 4-hydroxy proline, and δ- hydroxy lysine that involve essentially the functionalization of an inactive position. That these residues arise strictly by post

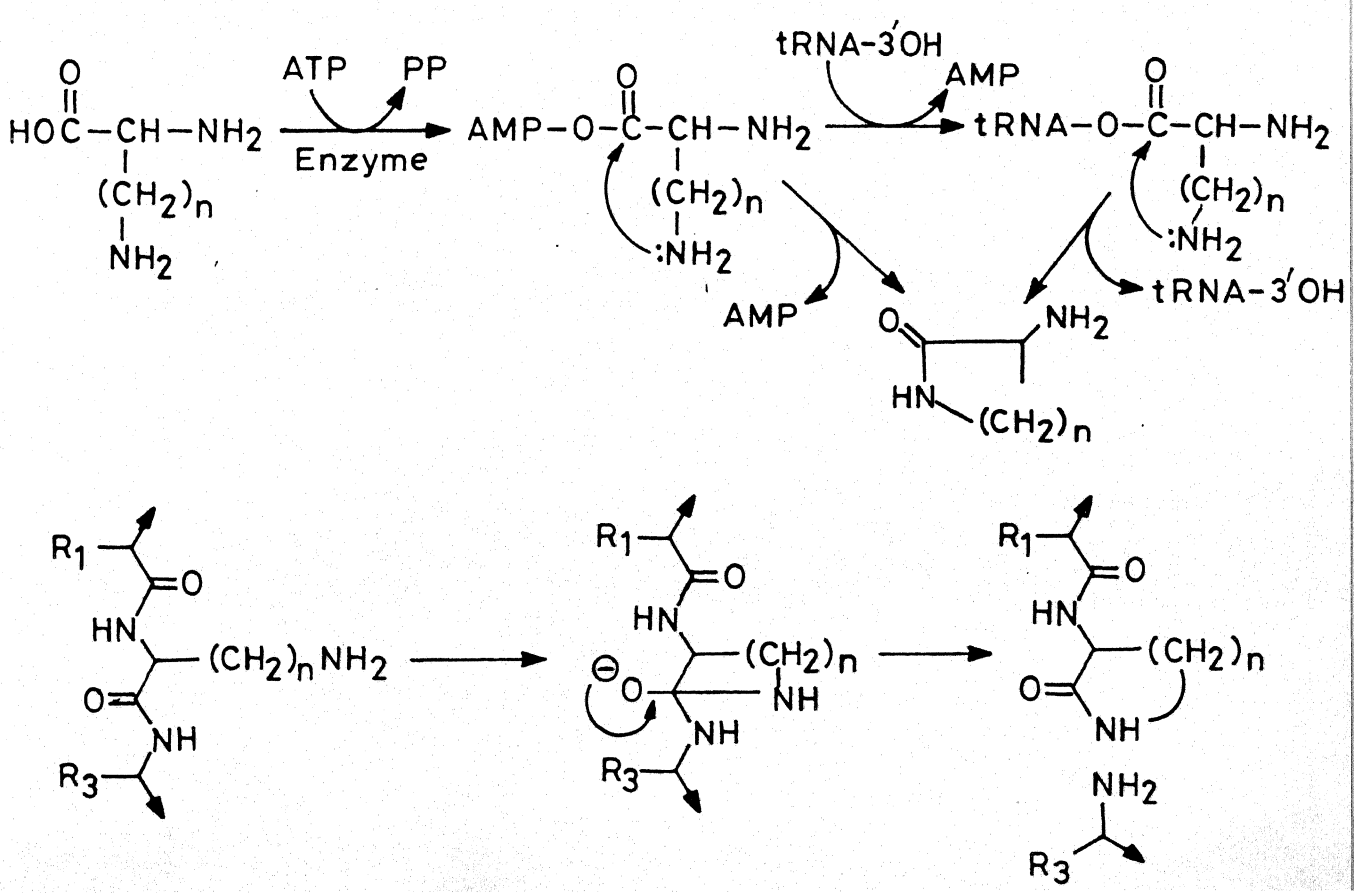
translational processes, is closely demonstrated by the fact that, the monomeric amino acids, namely, 4-hydroxyproline and δ-hydroxylysine, are not incorporated into the nascent proteins. A solution to the chemical simulation of these post translational processes would be along the lines of the transformation of papain to flavopapain involving the attachment of an appropriate flavin pendant on to a specific cysteine site, that is crucial for the proteolytic activity[36]. Apart from the fact that this operation enables the transformation of a protiolytic enzyme to one that can promote electron transfer reactions (CHART-C-IV), the major significance is that, it is possible to convalently attach appropriate pendants to side chains of complex proteins. This approach has been attempted to chemically simulate the post translational lysine hydroxylation.

CHART-C-IV



FLAVO PAPAIN (FIP)

CHART-C-V



THE TRANSFORMATIONS OF CHAIN SHORTENED LYSINE ANALOGS GENERATED FROM L-GLUTAMINE AND L-ASPARAGINE:

The non-inclusion of chain shortened analogs of lysine in the functional system could be as a result of two factors. Any inclusion in the translation setup should involve the charging of the appropriate t-RNA, with the amino acid. This results in the formation of an ester involving the 3'-hydroxyl group of the t-RNA. In principle, chain shortened lysine analogs could prevent this as a result of intramolecular cyclizations leading to cyclic lactams, thus making t-RNA attachment not possible (CHART-C-V). It is also possible that the precursor amino acid adenylate, could itself break down into the cyclic lactam. Either of these would prevent formation of viable t-RNA charged with the amino acid. Alternately, the lower homologs of lysine could destabilize proteins arising from translation via intramolecular attack, resulting in the rupture of the peptide bond (CHART-C-V).

To determine aspects that prevent lower homologs of lysine being part of the functional system, it was felt that N-benzyloxycarbonyl glutamine esters(1) and C-protected dipeptides having N-benzyloxycarbonyl glutamine residue (2), are appropriate sustrates, and PhI $(TFA)_2$ the reagent to bring about the desired $CH_2CH_2CONH_2 \dashrightarrow CH_2CH_2\ NH_2$ change.

$H_2NCOCH_2CH_2$-CH(NHZ)-COOR $H_2NCOCH_2CH_2$-CH(NHZ)-CONHCH-(X)-COOMe

(1) (2)

Cyclization of the ω-aminoethyl compound derived from (1), would reflect inability to be useful in translation, and that initiated by $H_2NCH_2CH_2$, arising from modification of (2), would show that, proteins carrying this unit can not survive and at the same time provide a method for specific protein cleavage at the glutamine site. Finally, the absence of rupture of the peptide bond, would provide a methodology for the preparation of modified proteins carrying an aminoethyl side chain in place of glutamine.

N-benzyloxycarbonyl glutamine, prepared by a modified procedure from the amino acid and benzyloxycarbonyl chloride, on treatment with diazomethane, gave, (1a), with p-nitrophenol/DCC,(1b) and p-nitro benzyl bromide, (1c). The reaction of Z-glutamine p-nitrophenyl ester (1b) with, respectively, glycine methyl ester, phenylalanine methyl ester, and leucine methyl ester, gave the dipeptides(2a),(2b) and(2c) (CHART-C-VI).

(1a) : mp. 138° C

IR : ν_{max} (KBr) cm^{-1} : 3440, 3340 (-NH), 1750(ester),1650,1550(amide).

NMR : δ($CDCl_3$+DMSO-d_6): 2.15(m,4H,-$C\underline{H}_2$,-$C\underline{H}_2$-CO-), 3.6(s,3H,-O$C\underline{H}_3$),4.5(m, 1H,tertiary proton), 5.0 (s,2H,-O-$C\underline{H}_2$-Ph),7.3(s,5H,aromatic).

(1b) : mp. 150°C.

(1c) : mp. 133°C.

IR: ν_{max}(KBr) cm^{-1} : 3500, 3350 (-NH), 1750(ester),1665,1620, 1540 (amide).

NMR: δ($CDCl_3$+DMSO-d_6): 2.28(m, 4H, - $C\underline{H}_2$-$C\underline{H}_2$-CO-), 4.2(m,1H, tertiary proton), 5.1(s,2H,-O-$C\underline{H}_2$-Ph),5.25 (s, 2H,-O-$C\underline{H}_2$-Ph(p-NO_2)), 7.3 -8.3 (m, 9H, aromatic).

2b : mp. 180°C.

IR : ν_{max}(KBr)cm^{-1} : 3440, 3320 (-NH), 1750 (ester), 1700 (sh), 1665, 1615 (sh), 1550 (amide).

NMR: δ ($CDCl_3$+DMSO-d_6): 2.15(m, 4H,- $C\underline{H}_2$-$C\underline{H}_2$-CO-), 3.0 (d,2H, J=7Hz, - $C\underline{H}_2$ Ph), 3.65 (s, 3H, - O-$C\underline{H}_3$), 4.2(m,1H, tertiary proton), 4.6(m,1H, tertiary proton), 5.0 (s, 2H, - O - $C\underline{H}_2$Ph), 7.15(m, 5H, aromatic), 7.25(s, 5H, aromatic).

ms: m/z: 441 (M^+).

(2a): mp. 172°C.

IR : ν_{max} (KBr) cm^{-1} : 3460, 3340 (-NH), 1750 (ester), 1665, 1615, 1555 (amide).

NMR: δ ($CDCl_3$+DMSO-d_6): 2.15 (m. 4H, $C\underline{H}_2$-$C\underline{H}_2$-CO-), 3.65 (s, 3H, O-$C\underline{H}_3$), 3.9 (d, 2H, J=7Hz, -NH-$C\underline{H}_2$-CO-), 4.2 (m,1H, tertiary proton), 5.05 (s, 2H, -O-$C\underline{H}_2$-Ph), 7.3 (s, 5H, aromatic).

(2c) : mp. 162°C.

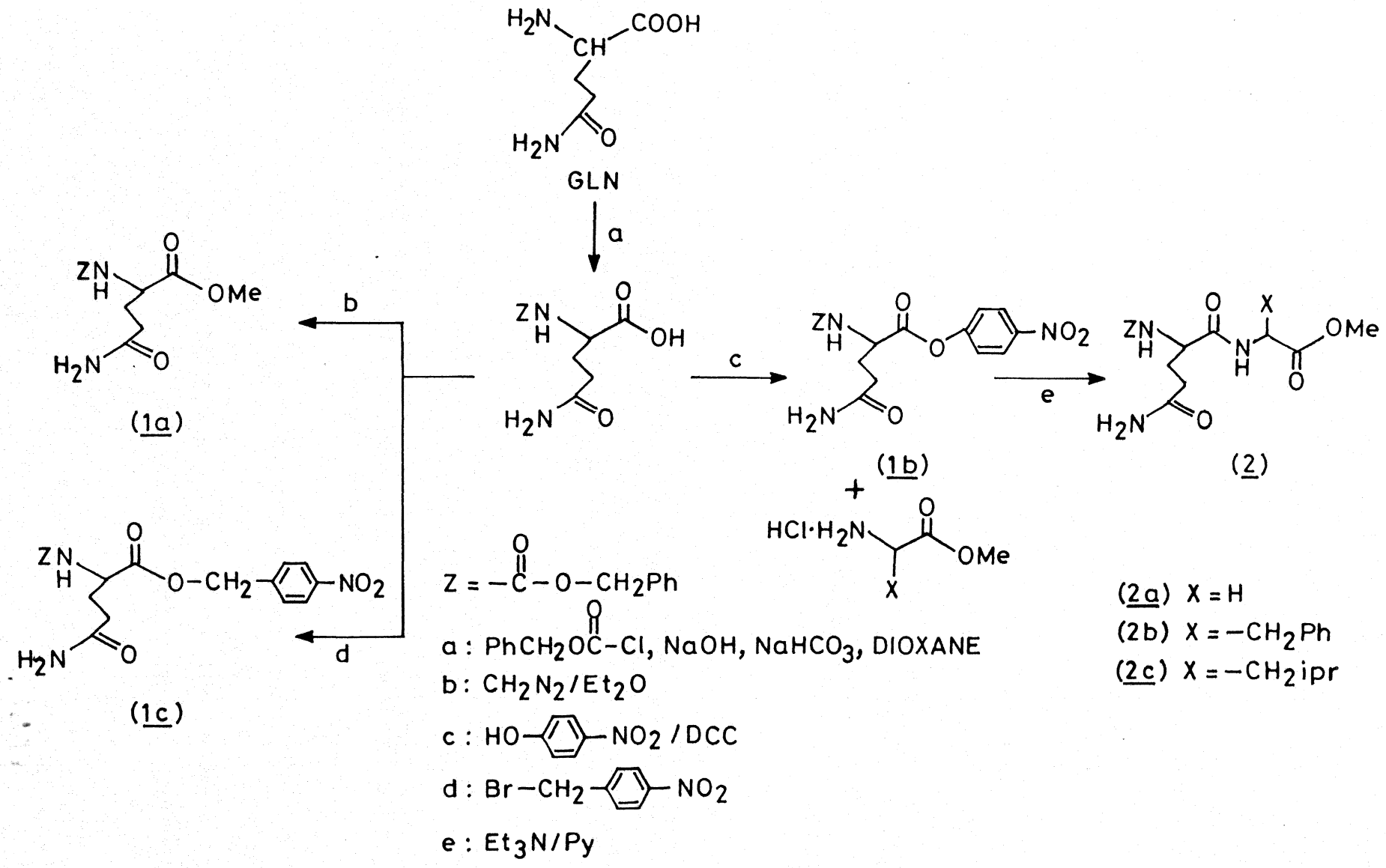
IR: ν_{max} (KBr) cm^{-1}: 3460, 3340 (-NH), 1740 (ester), 1690 (sh), 1665, 1620 (sh), 1550(amide).

NMR: δ ($CDCl_3$+DMSO-d_6): 0.95 (d, 6H, - $CH(C\underline{H}_3)_2$), 1.6 (m, 3H, - $C\underline{H}_2$-$C\underline{H}(CH_3)_2$), 2.15 (m,4H, - $C\underline{H}_2$-$C\underline{H}_2$-CO-), 3.65 (s, 3H, - O - $C\underline{H}_3$),4.2 (m, 2H, tertiary protons), 5.05 (s, 2H, - O - $C\underline{H}_2$Ph), 7.3 (s, 5H, aromatic).

ms : m/z : 407 (M^+) .

The reaction of N-benzyloxycarbonyl gulutamine esters (1a), (1b) and (1c), with PhI $(TFA)_2$ in aq. CH_3 CN at rt. for 3h, resulted in the expected $CONH_2$-→NH_2 degradation, and when the resulting salts were left strirred with aq. $NaHCO_3$ - CH_2 Cl_2 for 3h, underwent spontaneous cyclization in each case giving rise to

CHART-C-VI


GLN
a
b
c
d
e
(1a)
(1b)
(1c)
(2)
Z = −C(=O)−O−CH2Ph
a : PhCH2OC(=O)−Cl, NaOH, NaHCO3, DIOXANE
b : CH2N2/Et2O
c : HO−C6H4−NO2 / DCC
d : Br−CH2−C6H4−NO2
e : Et3N/Py
(2a) X = H
(2b) X = −CH2Ph
(2c) X = −CH2ipr

cyclic lactam (3)[37], in respectively, 55%, 70%, and 45%[38] yields, (CHART-C-VII).

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(3) mp. 175°C.

IR: ν_{max} (KBr) cm^{-1} : 3270 (-NH), 1670, 1650, 1540 (amide).

NMR: δ ($CDCl_3$ + DMSO-d_6): 3.30(m, 4H, - CH_2-CH_2-NH), 4.2 (m, 1H, tertiary proton), 5.05 (s, 2H, -O-CH_2-Ph) 7.35 (s, 5H, aromatic).

ms: m/z : 234 (M^+).

The spontaneous cyclization observed leading to (3) in good yields, clearly shows, that α - amino acids having the end group NH_2 spaced 2-methylenes away cannot be involved in the translation protocol, since they would not permit the realization of any of the cruicial stages involved leading to the charging of this residue to appropriate t-RNA.

Further evidence to this was obtained from similar studies on dipeptides (2). The glutamine side chain of (2a), (2b) and (2c), were effectively degraded with PhI $(TFA)_2$ in aq. DMF, and the resulting amino compounds (4a), (90%), and (4b), (74%), characterized (CHART-C-VIII).

4a: IR: ν_{max} (thin film) cm^{-1} : 3320 (-NH), 1750 (ester), 1690 (br), 1550 (amide), 1540.

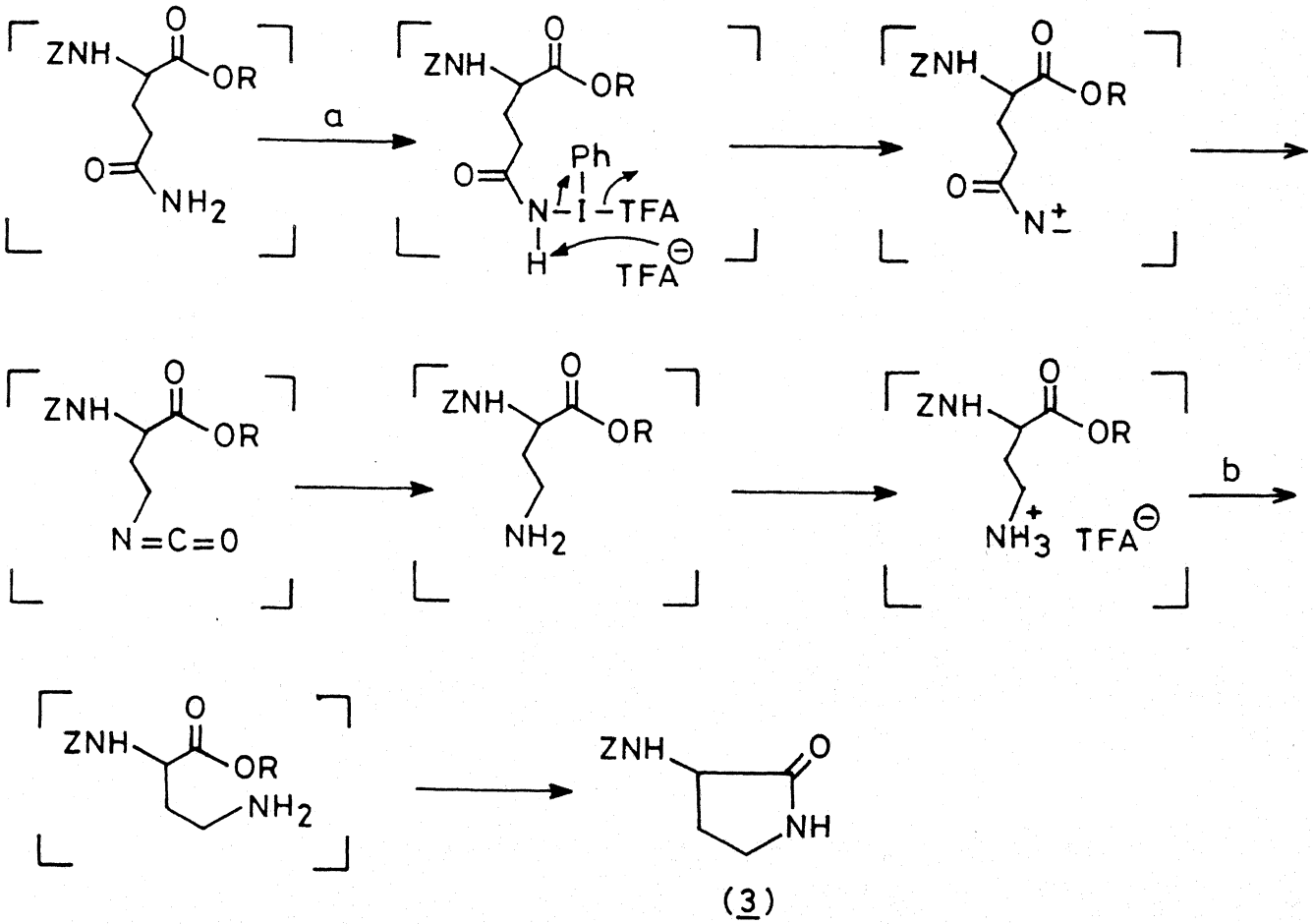
NMR: δ ($CDCl_3$) : 2.6 - 3.2 (m, 4H, $C\underline{H}_2$ - $C\underline{H}_2$-NH_2), 3.6 (s, 3H, -O-$C\underline{H}_3$), 4.0 (m, 2H, tertiary protons) , 5.10 (s, 2H,-O-$C\underline{H}_2$-Ph), 7.25 (s, 5H, aromatic).

4b : IR: ν_{max} (thin film) cm^{-1} : 3440, 3320,(-NH), 1750 (ester), 1690, 1610, 1515 (amide).

NMR: δ ($CDCl_3$): 2.9 (m, 6H,-$C\underline{H}_2$-$C\underline{H}_2$-NH_2) and $C\underline{H}_2$-Ph), 3.61 (s, 3H, -O-$C\underline{H}_3$), 4.4, (br, 2H, tertiary protons), 5.0 (s, 2H, -O-$C\underline{H}_2$-Ph), 7.05, (s, 5H, aromatic), 7.25 (s, 5H, aromatic).

Endeavours to demonstrate the side chain mediated rupture of the peptide bond in (4a), (4b) or (4c), that would have resulted in the formation of lactam (3), under conditions of (1)-- (3), change as well as at pH 7 (phosphate buffer), in aq. $NaHCO_3$, in aq. MeOH-$NaHCO_3$, and in MeOH-$NaHCO_3$, failed .

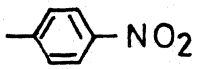
The demonstrated stability of (4) under conditions similar to that which exist in biological systems, is particularly noteworthy. Apart from clearly demonstrating that the reason for non-selection of chain shortened lysine analogs in the coded



a : $PhI(TFA)_2$, Py , Aq. CH_3CN

b : Aq. $NaHCO_3$, CH_2Cl_2

(1a) : R = CH_3

(1b) : R = 

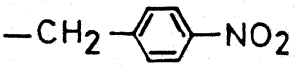
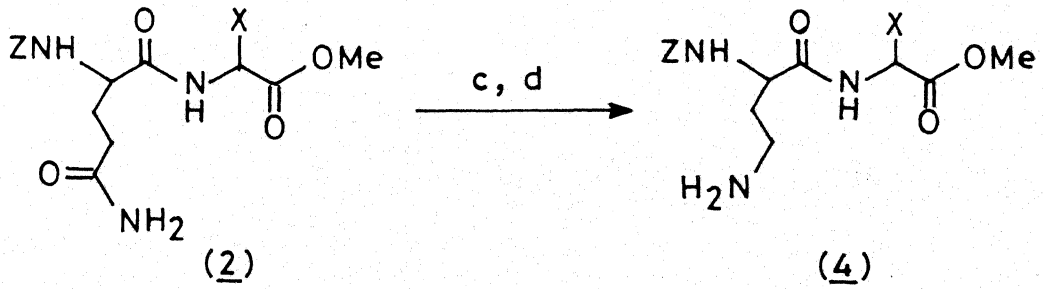
(1c) : R = 

CHART-C-VIII



(2a) : X = H C: $PhI(TFA)_2$, Py, Aq. DMF

(2b) : X = CH_2Ph d: HCO_3^-

(2c) : X = CH_2ipr

systems is solely due to their inability to take part in the normal operations leading to protein synthesis, these results also show that where NH_2-CH_2-CH_2- side chain be present in proteins, either from degradation of a glutamine precursor such as in the present work, or by other possible methods, they would not destabilize the molecule. This finding should logically lead to a wide spectrum of studies, with focus on enzymes which are know to have a lysine residue at the active site.

Asparagine would give rise to, by the chain shortening process, a system, where the terminal amino function is separated from the - amino acid noiety by a singly α-CH_2 grouping. In the event, the reaction of Z-Asn-OMe (5), prepared via procudure similar to that for (1a) (CHART-C-VI), with $PhI(TFA)_2$ in aq.CH_3CN, proceeded smoothly to give the expected amino compound (6), (vide infra). Although it was considered unlikely that (6) would cyclize to give the -lactam, efforts were made in this direction without success. The free base arising from (6), unless used immediately, decomposes to complex mixtures. It was then envisaged that the terminal NH_2 group in (6) could, in turn, generate an appropriately positioned amino function, which would then interact with the ester grouping leading to cyclization. This was realized. The reaction of insitu generated (6), with ethyl isothiocyanate, gave the novel cyclized product (8), (20%), and the open adduct (7a), (41%). It was further demonstrated that the open adduct (7a), cyclizes to (8), on refluxing in benzene. In the case of PhNCS, the reaction at rt stops at the

open adduct stage (7b) which was isolated in 63% yields, thus again demonstrating that the expected (5) -->(6) has taken place in good yields. Cyclohexyl isothiocyanate, in a similar manner, gave the adduct (7c) in (20%) yields [39] (CHART-C-IX).

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(5) : mp 150°C

IR: ν_{max} (KBr) cm^{-1} : 3440, 3320, (-NH), 1750 (ester), 1685, 1560 (amide).

NMR:δ($CDCl_3$ + DMSO-d_6): 2.7(d, 2H, J=7Hz,-$C\underline{H}_2$-CO-), 3.65(s, 3H, O-$C\underline{H}_3$), 4.5 (m, 1H, tertiary proton), 5.05 (s, 2H, O-$C\underline{H}_2$-Ph), 7.3 (m, 5H, aromatic).

(7a): IR: ν_{max}(thin film) cm^{-1}: 3440, 3340 (-NH), 3000, 1720(ester), 1540 (amide).

NMR: δ ($CDCl_3$): 1.20 (t, 3H,-CH_2-$C\underline{H}_3$), 3.35 (m, 2H, -$C\underline{H}_2$-CH_3), 3.7(s, 3H, -O-$C\underline{H}_3$) , 3.95 (m, 2H, -$C\underline{H}_2$-N-), 4.45 (m, 1H, tertiary proton), 5.05 (s, 2H,-O-$C\underline{H}_2$-Ph), 7.3(s, 5H, aromatic).

ms: m/z : 339 (M^+).

(7b): mp. 122°C.

IR: ν_{max} (KBr) $cm.^{-1}$: 3380, 3320, 3220 (-NH), 1730 (ester), 1605, 1555 (amide).

NMR: δ ($CDCl_3$): 3.7 (s, 3H, -O-CH_3), 4.1 (m, 2H, -CH_2-), 4.5(m,1H, tertiary proton), 5.0 (s, 2H, -O-CH_2-Ph), 7.25(m, 10H, aromatic).

ms: m/z: 387(M^+).

(7c): mp. 126-28°C.

IR: ν_{max} (KBr)cm^{-1}: 3370 (-NH), 2960, 2880, 1740 (ester), 1710, 1565, 1545 (amide).

NMR: δ ($CDCl_3$): 1.2-2.0 (m, 11H, cyclohexyl protons), 3.75 (s, 3H, -O-CH_3), 3.9 (m, 3H, -CH_2- and - NH-CH-), 4.40 (m, 1H, tertiary proton), 5.05 (s, 2H, -O-CH_2-Ph), 7.3 (s, 5H, aromatic).

ms: m/z : 393 (M^+).

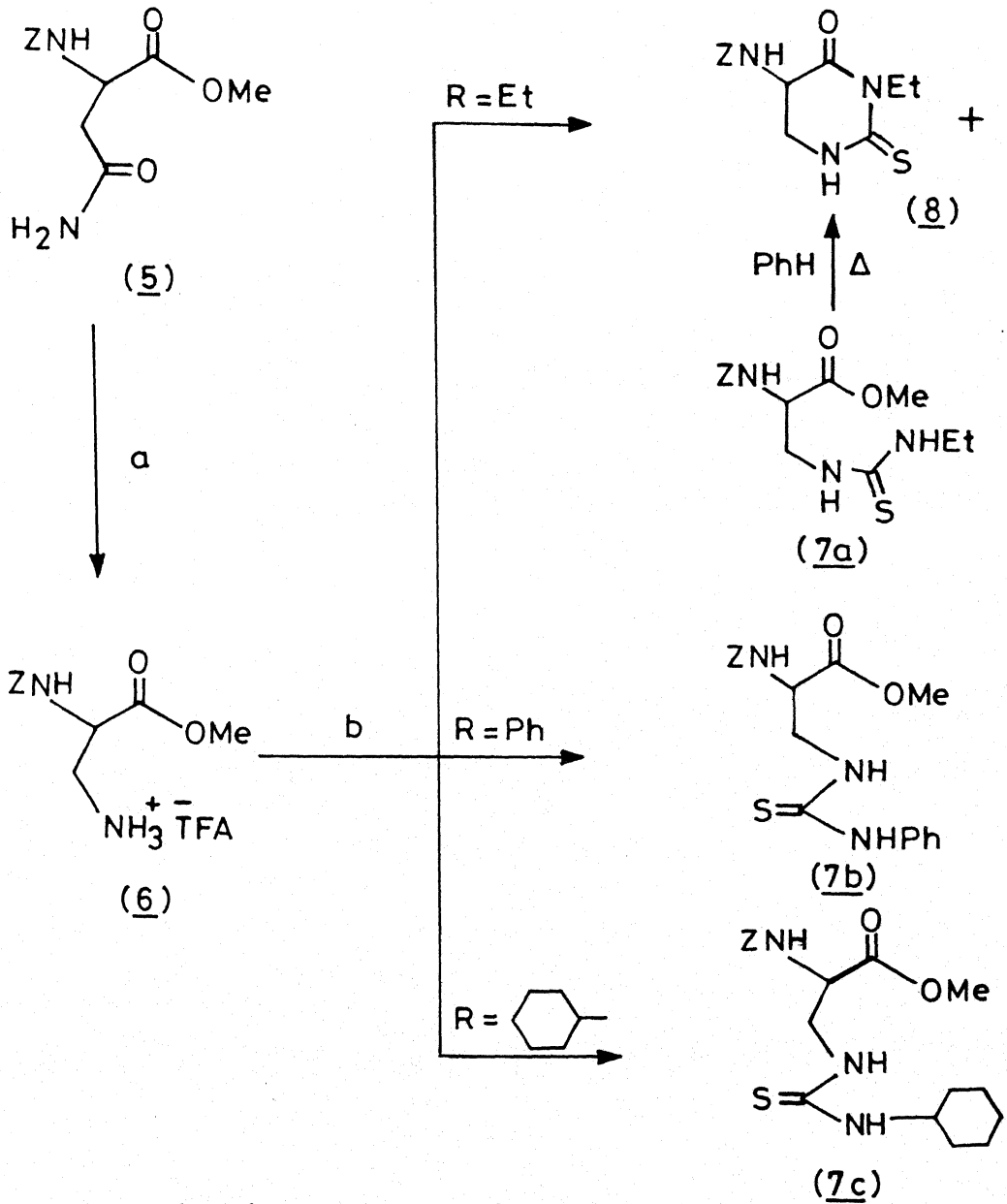
---

(8) : mp. 108°C.

IR: ν_{max} (KBr) cm^{-1} : 3320 (-NH), 3190, 1710, 1560, 1530.

NMR: δ ($CDCl_3$):1.2(t, 3H, -CH_2-CH_3), 3.65-4.85 (m,5H,-CH_2-CH_3, -CH_2-N-, and tertiary proton), 5.10 (s, 2H, -O-CH_2-Ph),

CHART-C-IX



a : $PhI(TFA)_2$, Py, Aq CH_3CN

b : Et_3N, CH_2Cl_2, RNCS

7.3(s,5H, aromatic).

ms: m/z: 307 (M^+).

$[\alpha]_D^{25} = -2.4^\circ$.

The dihydrouracil (8) has several interesting and useful facets. It is optically active, can be quite easily made, provides, in principle, opportunities for the preparation of diverse 3- substituted analogs by use of the appropriate isothiocyanate, and the thioamide unit present, should enable the selective attachment of sugar residue to give novel nucleosides and nucleotides.

As stated earlier, the spacers appropriate to the coded amino acids play a very important role (CHART-C-III). In the event, the end group is the same, the homologs arising by mere change in number of spacer methylenes would show a gradation in reactivity. In the present work, this aspect finds exprression in the sense that, in striking contrast to the smooth degradation of the glutamine dipeptide (2b), the asparagine analog (9), under the same conditions, was hardly touched.

$H_2NCOCH_2CH(NHZ)CONHCH(CH_2Ph)COOMe$

(9)

---

(9) : mp. 198°C.

IR: ν_{max}(KBr) cm^{-1}: 3440, 3300 (-NH), 1750 (ester),

1700, 1640, 1550 (amide).

NMR: δ ($CDCl_3$+DMSO-d_6): 2.05 (d, 2H, $C\underline{H}_2$-CO-), 3.0 (d, 2H,-$C\underline{H}_2$-Ph), 3.65 (s, 3H, -O-$C\underline{H}_3$), 4.5(m, 2H, tertiary protons), 5.0 (s, 2H, -O-$C\underline{H}_2$Ph), 7.15(s, 5H, arometic), 7.3 (s, 5H, arometic).

ms: m/z : 427 (M^+).

This profound difference in reactivity is fortunate, since it would enable the specific degradation of glutamine side chains in peptides without affecting asparagine residue that would have led to complications arising from the presence of unstable H_2NCH_2- units.

The differentiation of coded amino acid side chains carrying the same functional group and differing only in the number of spacers, such as that encountered in the present work (vide supra), could have practical potential as well. This aspect can be illustrated with ACTH sequence presented in CHART-C-X which shows that the difference between bovine ACTH and human ACTH is only with respect to the presence of a glutamine residue in the latter instead of the glutamic acid that is present in the former at position 33. Consequently, it should be possible to convert bovine ACTH to human ACTH via hydrolysis of glutamine residue at 33 position. The problem however would be the presence of the 21 asparagine in both the types, which should not be affected.

CHART-C-X

ACTH

HUMAN / BOVINE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
OVINE / PORCINE	S	Y	S	N	E	E	F	R	W	G	K	P	V	G	K	K	R	R	P	V

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
HUMAN	K	V	T	P	N	G	A	E	D	E	S	A	E	A	F	P	L	E	F
BOVINE													Q						
PORCINE											L								
OVINE	D												Q						

Thus, the bovine to human ACTH change would require hydrolysis of the glutamine residue to glutamic acid without affecting the asparagine present. The diference in reactivity observed in the present work between the above two residues should make, therefore, the chemical transformation of bovine ACTH to human ACTH possible.

STUDIES ON THE TRANSFORMATION OF GLUTAMINE TO HISTIDINE IN A PEPTIDE ENVIRONMENT

The special relationship that prevails between the histidine-glutamine pair in the coding system has already been mentioned (CHART-C-II and TABLE-C-I). On their own right, this pair plays perhaps a more crucial role in life systems than any other. Histidine is present in the active sites of almost all enzymes and glutamine is critical for nitrogen metabolism. The latent structural relationship between glutamine and histidine surfaces, when a retrosynthetic analysis is made as shown in (CHART-C-XI).

CHART-C-XI shows that glutamine could be transformed to histidine via incorporation of elements of formamide. This can be, as shown , accomplished in two different ways, either by addition to an eynamine unit generated from glutamine, or via attachment of this residue to the δ-carbon, leading to δ-formamido glutamine. Most interestingly, the δ- formamido glutamine unit is encountered in the course of metabolism of histidine, in bacteria and liver (CHART-C-XII[40]), and can be realized chemically via oxidation of N, C protected histidine with 4-^{t}-butyl iodoxybenzene [41], (CHART-C-XIII). It could be seen from these charts, that both the processes involve the histidine hydroxylation at 4-position, prototropic shift, and

CHART-C-XI

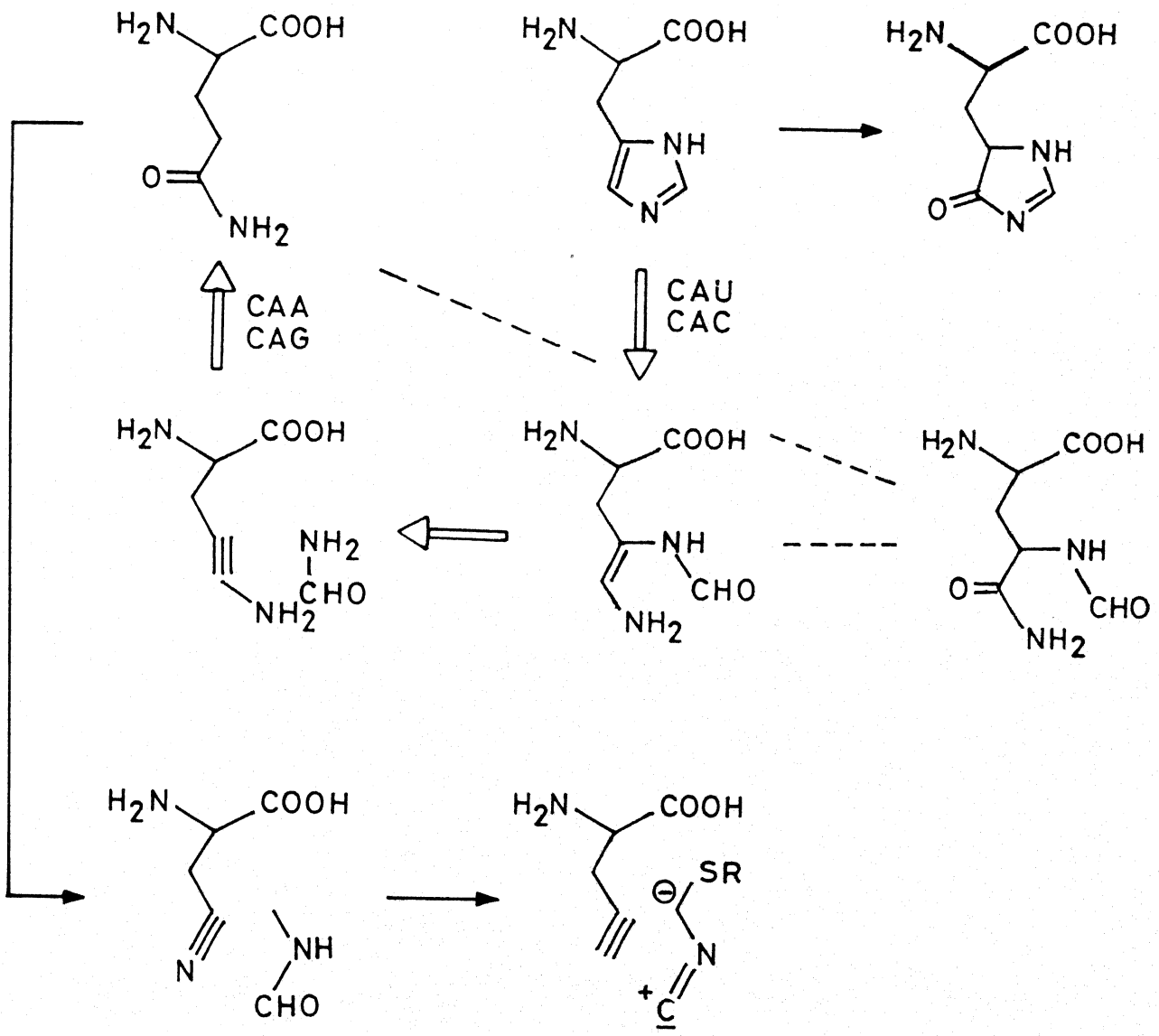
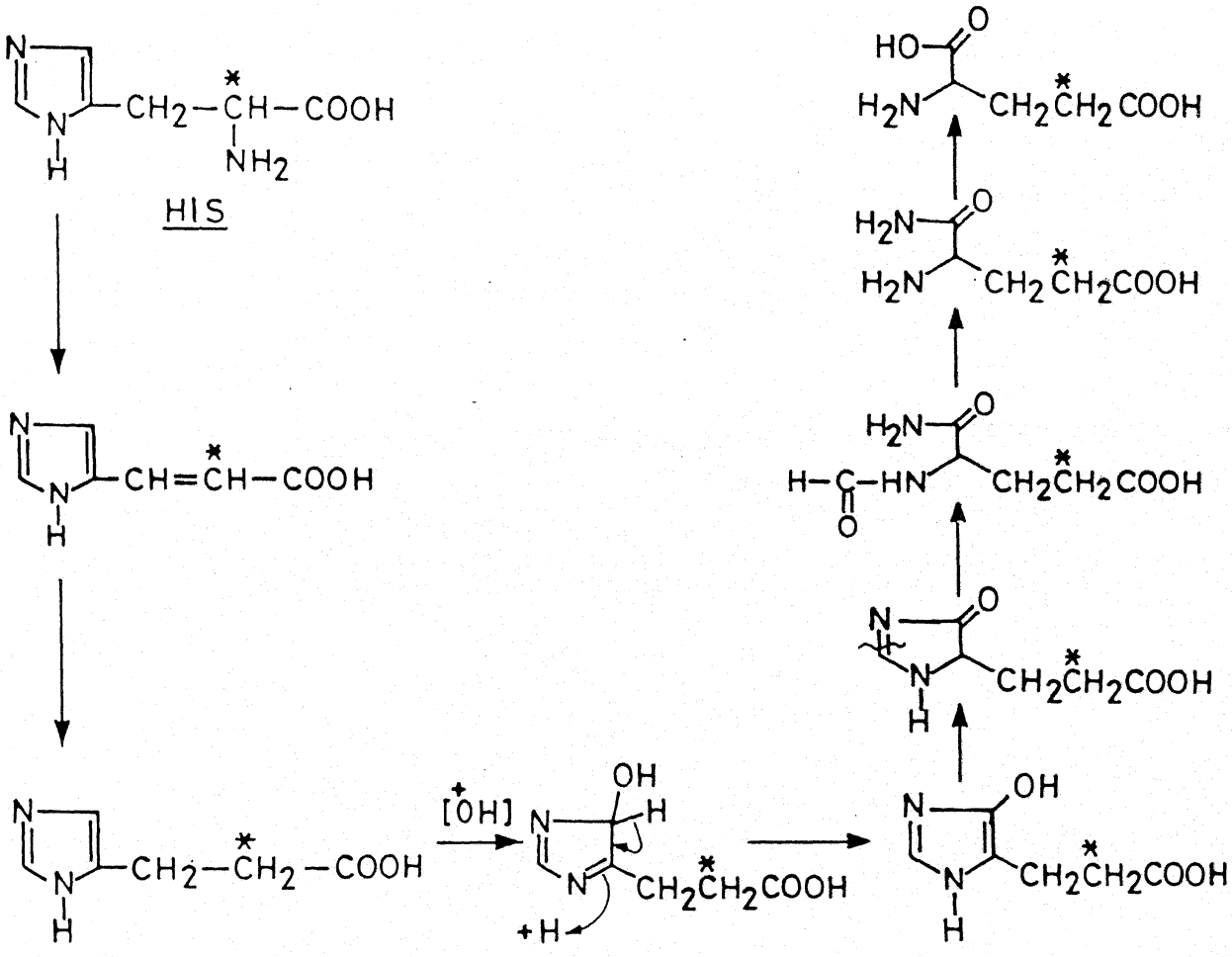


CHART-C-XII



cleavage. The formamidoglutamine route to histidine should therefore involve a reduction (CHART-C-XI).

In the present work, the transformation of the glutamine side chain to that of histidine presented an attractive objective for several reasons. The obvious is the development of methodology for creating an imidazole system from an amide linkage. The potential use of such a transformation in peptides, leading to the replacement of the glutamine side chain with that of histidine, would have profound application .

Additionally, in the synthesis of peptides, some amino acid side chains create complications when carried over a number of steps, and one of these is histidine. Such complications, in principle, could be obviated by using a histidine equivalent, which after the synthetic operation, could be transformed to the desired system. Glutamine would be a good choice. The strategy that was considered feasible was a modified scheme that is presented in retrosynthetic analysis (CHART-C- XI).

It was considered practical to degrade glutamine to a lower nitrile and then to generate the imidazole ring via cycloaddition to a N-methyl formamide equivalent. Specifically then, the task involved the transformation of the glutamine to a β-cyanoalanine, and subject this to cycloaddition with the conjugate base of aryl thiomethyl isocyanide (CHART-C-XI).

The first objective relating to the glutamine-->histidine transformation, namely, the degradation of glutamine side chain, was accomplished in a novel manner.

N-benzyloxycarbonyl glutamine methyl ester (1a) was degraded to the amine-TFA salt as described earlier (CHART-C- VII) and then directly transformed to the desired nitrile (10), via treatment with t-BuOCl[42] and pyridine (CHART-C- XIV).

---

(10) : mp, 89°C.

IR: ν_{max} (KBr) cm^{-1} : 3350 (-NH), 2290 (-C≡N), 1750 (ester), 1700, 1560 (amide).

NMR: $\delta(CDCl_3)$: 2.95 (d, 2H, -$C\underline{H}_2$-CN), 3.80 (s, 3H, -$OC\underline{H}_3$), 4.55 (m, 1H, tertiary proton), 5.10 (s, 2H, O-$C\underline{H}_2$Ph), 7.3(s, 5H, aromatic).

The structural assignment for (10) was fully confirmed by comparison with an authentic sample prepared from the dehydration of benyloxycarbonyl asparagine with DCC/Py, followed by esterification.

The facile trasnformation of (1a) -->(10) represents a novel method for the transformation of an amide to its lower nitrile. A number of methods are, of course, available for the dehydration of a carboxamide to the corresponding nitrile without

CHART-C-XIII

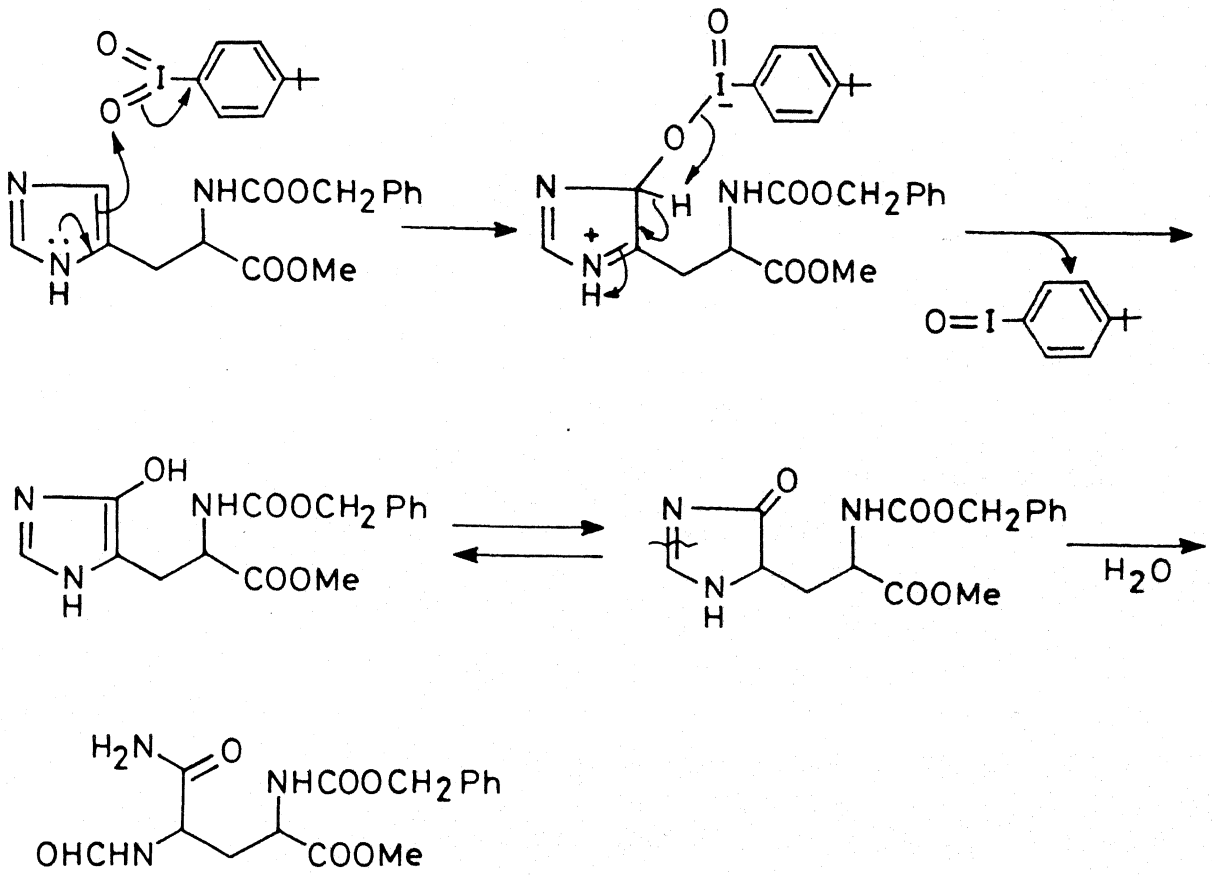
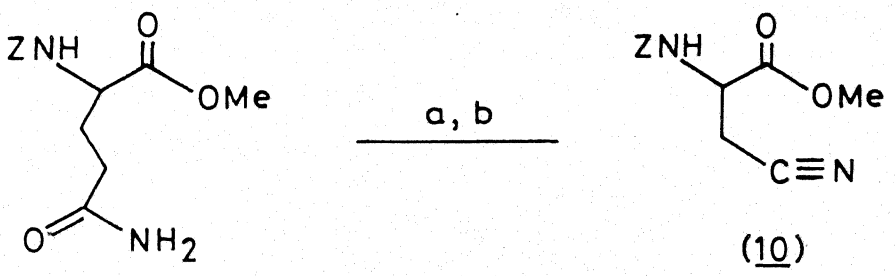


CHART-C-XIV



(1a) (10)

a : $PhI(TFA)_2$, Py , Aq CH_3CN

b : tBuOCl , Py

degradation. Since compound (10) can be easily transformed by alkaline H_2O_2 to benzyloxycarbonyl asparagine, the present procedure for the (1a) --→(10) change could be useful for the transformation of glutamine side chains to that of asparagine. As stated earlier, the asparagine side chains, if any, present in the protein, are expected to be unaffected by the PhI $(TFA)_2$ reagent.

The synthetic strategy for the glutamine--→histidine transformation envisaged the degradation of glutamine to a lower nitrile (-1), and attachment of one carbon residue to formamide (+1) (CHART-C- XI). Thus, further endeavours must involve the union of (10) with N-methylformaide. A careful examination of literature showed that there is only one methodology that could bring about the desired union, namely, cycloaddition of the conjugate base of aryl thiomethyl isocyanides followed by desulfurization.

The early phases of the work pertaining to the above goal brought out two major shortcomings in the existing procedures related to such cycloadditions. The most serious was the very obnoxious nature of either $PhSCH_2$-NC or pCH_3-C_6H_4-SCH_2NC. They were evil smelling liquids. In addition, their preparations involving multi-step processess were not practical. It was considered attractive to design an aryl thiomethyl isocyanide which would have a higher melting point and lower vapour pressure. The choice was 2-thionaphthylmethyl isocyanide (11). The preparation of (11) envisaged the transfer of a

formamidomethyl unit to 2-thionapthol. The needed transfer reagent N- (Formamido methyl)-N-benzyl morpholinium iodide (12) was prepared from morpholine in an overall yield of 64% (CHART-C-XV). The formamidomethyl transfer to 2-thionaphthol was achieved in 90% yields via reaction of (12) in dry benzene in the presence of Et_3N. The resulting (13) was converted to the desired isocyanide (11), mp. 75°C, in 80% yields (CHART-C- XV). This procedure for (11) from the overall view point of yield, simplicity, convenience and workup is most attractive. 2-Thionaphthylmethyl isocyanide (11) is totally devoid of the highly disagreeable and pervasive odour associated with the earlier isocyanides.

---

(11): mp. 75°C.

IR : ν_{max} (KBr) cm^{-1} : 2140 (-NC).

NMR: δ ($CDCl_3$), 4.6 (s,2H, -S-C$\underline{H}_2$), 7.4-8.1(m,7H,aromatic).

ms: m/z : 199 (M^+).

---

(12) : mp. 156°C.

IR : ν_{max}(KBr) cm^{-1}:3380, 3210, 3050, 1715, 1685, 1545, 1530.

---

(13) : mp. 63°C.

IR: ν_{max}(KBr)cm^{-1}: 3310(-NH), 1680, 1530 (amide).

NMR: $\delta(CDCl_3)$ 4.5 (d, J=7Hz)+4.7(d,J=7Hz), 2H,-S-CH_2-, in the ratio 7.5 : 92.5, possibly arising from HN-CHO π barrier, 6.23 (br, 1H,-N<u>H</u>), 7.3-8.1 (m, 8H, aromatic).

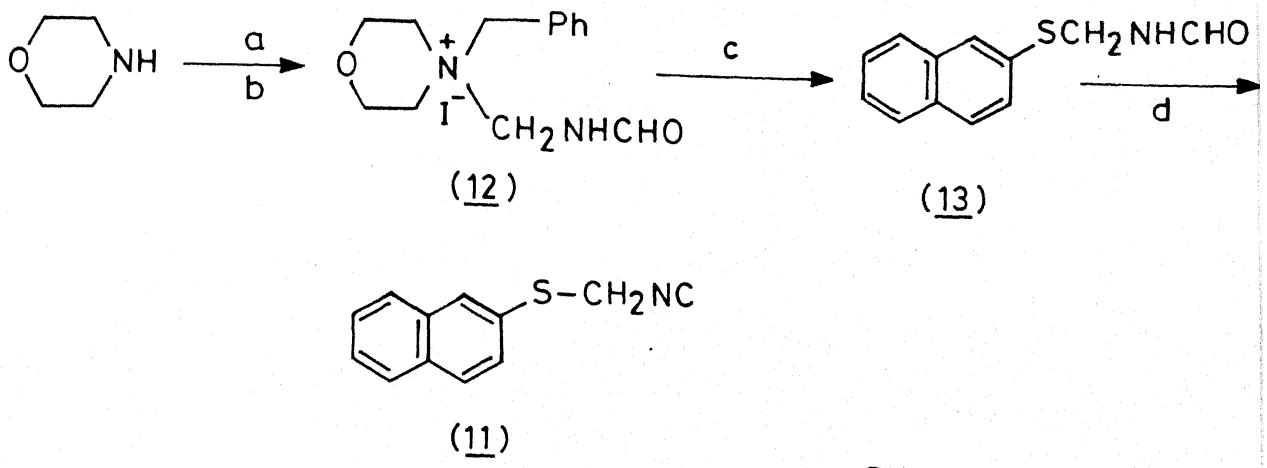
ms: m/z : 217(M^+).

Transfer reagents that are capable of transfering the desired synthon unit to the appropriate substrate, have found valuable application in organic synthesis, since reactions thus patterned are often clean and shorten synthetic procedures by several steps. In this context, the novel transfer reagent <u>(12)</u> should find applications in organic synthesis. Preliminary studies have been carried out to assess the potential of <u>(12)</u> for transfer of elements of CH_2NHCHO.

The reaction of <u>(12)</u> with 2-thionaphthol, leading to <u>(13)</u>, could , in principle, proceed by two distinct pathways, namely, via direct nucleophilic displacement by $Ar\bar{S}$, or by the fragmentation of <u>(12)</u> to the highly electrophilic $H_2C{=}\overset{+}{N}HCHO$ and Michael addition involving $Ar\bar{S}$, (CHART-C-XVI). It was hoped that a study of the reaction of <u>(12)</u> with diverse acceptors would help in the delineation of the most probable pathway pertaining to the formation of <u>(13)</u> from <u>(12)</u>.

The transfer to PhSH from <u>(12)</u> takes place readily under conditions of the <u>(12)</u>→ <u>(13)</u> change leading to <u>(14)</u> in excellent

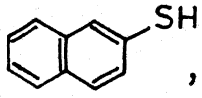
CHART-C-XV



(12) (13) (11)

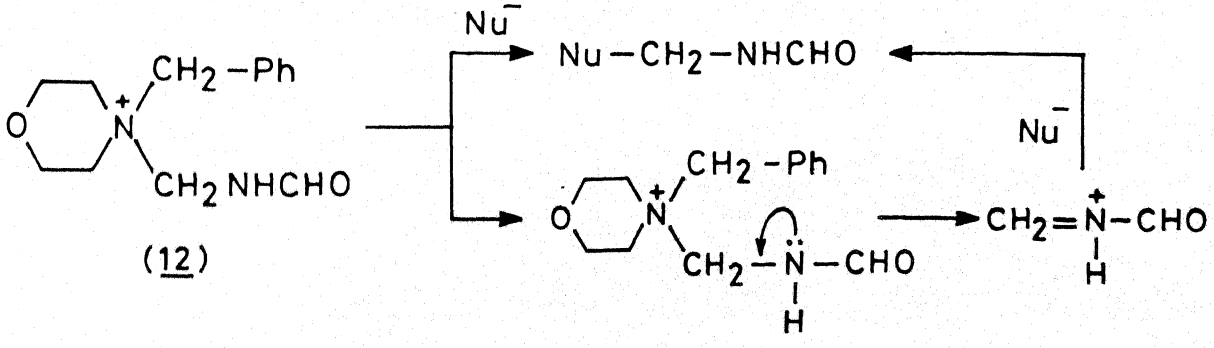
a : Formalin , $HCONH_2$

b : $PhCH_2I$

c :  , Et_3N

d : PPh_3, CCl_4, Et_3N, $CHCl_3$

CHART-C-XVI



(12)

yields. In terms of yields and simplicity, this transformation is the most convenient for the preparation of (14) since it obviates complexities arising from operating in polar media such as methanol and the use of strong bases like sodiam alkoxide, used in earlier procedures[44].

---

(14): mp. 32°C.

IR: ν_{max}(thin film)cm^{-1}: 3320 (-NH), 1690, 1540 (amide).

NMR: $\delta(CDCl_3)$: 4.6 (d, J=7Hz, 2H,-S-C$\underline{H}_2$), 7.3 (m, 6H, aromatic), 7.9 (br, 1H,-C$\underline{H}$O).

Benzaldehyde oxime presents an interesting profile for transfer studies with (12), in the sense that, in principle, it has three nucleophilic centres (O, N, C) to initiate the transfer. It was also envisaged that the site of transfer could be modulated by choice of the medium. In the event, the reaction of (12) with benzaldehyde oxime, either under benzene/Et_3N reflux, or reflux in MeOH/NaOMe, gave the O-formamidomethyl benzaldoxime (15) in respectively 61% and 76% yields.

---

(15) : mp. 60°C.

IR: ν_{max}(KBr) cm^{-1}: 3290 (-NH), 1660, 1520 (amide).

NMR: $\delta(CDCl_3)$: 5.22 (t, 2H,-OC$\underline{H}_2$- NH), 6.7 (br, 1H,-

NH),7.3-7.7 (m, 5H, aromatic),8.1(d, 1H), 8.25 (s, 1H).

ms: m/z: 178 (M^+).

The structural assignment for (15) is further supported by its transformation with 2, 4-DNP reagent to benzaldehyde-2, 4-dinitrophenyl hydrazone, either directly, or after $TiCl_3$ cleavage.

Endeavours to transfer CH_2NHCHO to either 2-naphthol or phenol gave surprising results. In neither case was the transfer to either oxygen or carbon observed. In the case of 2-naphthol, the dimeric product (16) was isolated with reagent (12), both in benzene/ Et_3N (40%) and MeOH/NaOMe (74%). In a similar manner, indole in DMSO, on treatment with (12) without any base, was transformed to dimer (17) in 20% yields.

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(16) : mp. 196°C.

IR: ν_{max} (KBr) cm^{-1}: 3360 (-OH), 1640, 1620.

ms: m/z : 300 (M^+).

---

(17): mp. 162°C.

IR: ν_{max} (KBr) cm^{-1}: 3420, (-NH), 1625, 1605, 1460.

NMR: δ ($CDCl_3$): 4.2 (s, 2H, -$C\underline{H}_2$), 6.9-7.6 (m, 12H, aromatic).

ms: m/z: 246 (M^+).

The reaction of phenol with (12) gave results which provided a clue to the genesis of (16) and (17). Although the reaction between phenol and (12) in MeOH/NaOMe was complex, the 2-morpholinomethyl mannich base (18) could be isolated in 10% yields, (CHART-C- XVII).

---

(18): mp:90°C.

IR: ν_{max} (KBr) cm^{-1} : 1625, 1600, 1500, 1470.

NMR: δ ($CDCl_3$): 2.5 (m, 4H, morpholine protons), 3.7 (m, 6H, morpholine protons), 6.6-7.4(m,4H, aromatic), 8.3 (br, 1H, O$\underline{H}$).

ms: m/z: 193 (M^+).

The spectrum of products obtained mediated by the transfer reagent (12) can be rationalized on the basis of an integrated mechanism presented in (CHART-C- XVIII).

The formation of (18) can be explained only on the basis of the reactive intermediate (B) (CHART-C- XVIII) which, in turn , will arise via intermediate involving nucleophilic displacement of the CH_2 Ph moiety. The fact that the dimeric products (16) and (17) are formed in good yields and whose formation also is

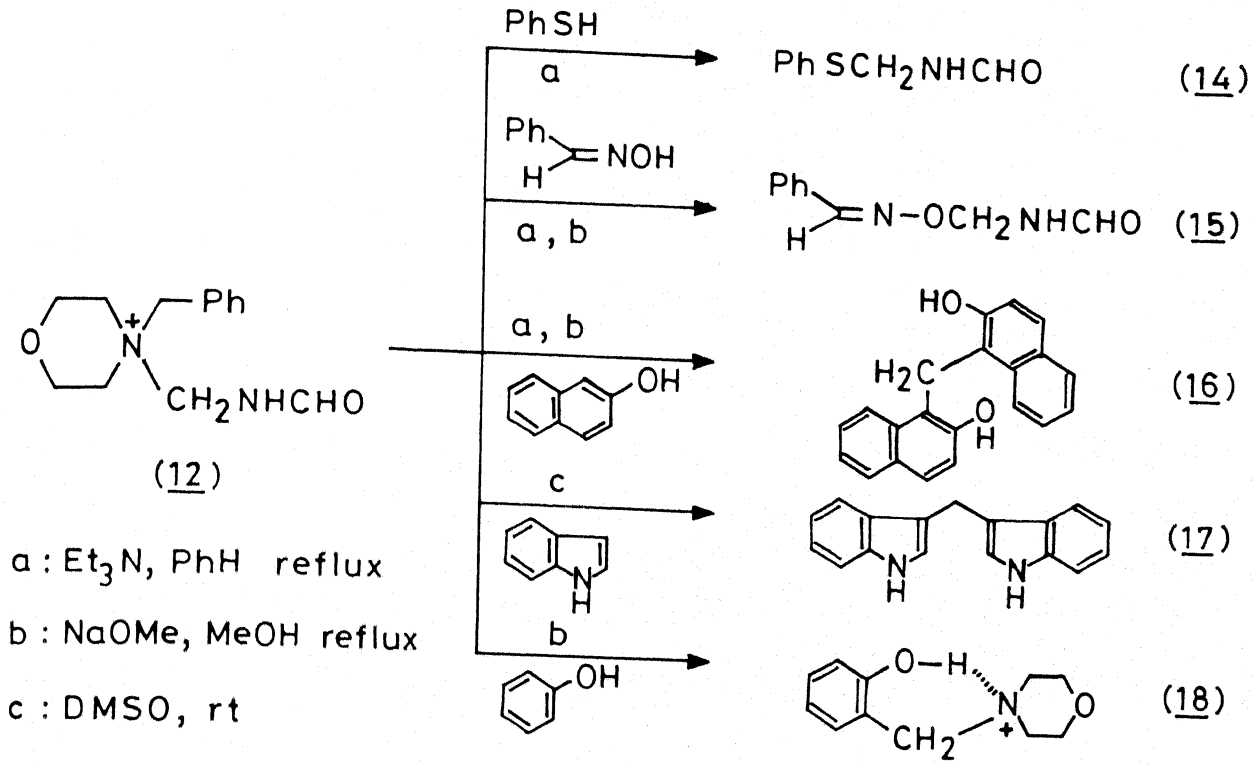
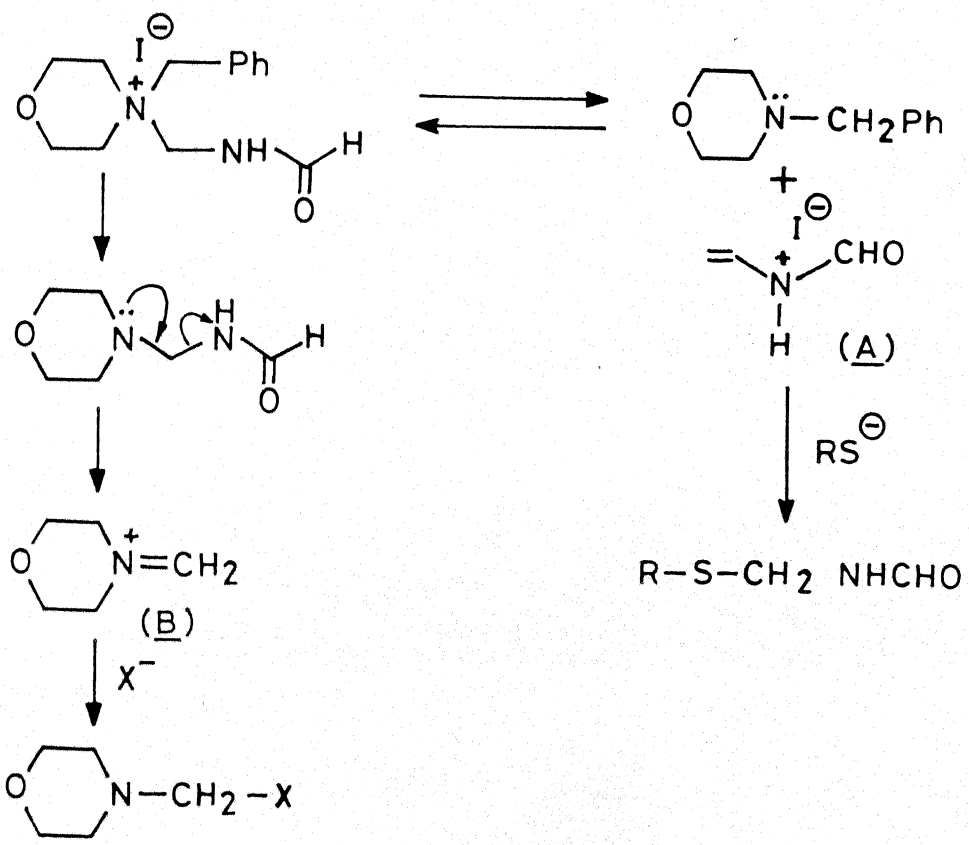


CHART - C - XVIII



best rationalized on the basis of (B) would mean that the proposed nucleophilic displacement is exclusively affecting the CH_2Ph moiety of (12). This would tend to support the view that the transfer products (13), (14) and (15) do not arise by direct displacement and that their formation is better rationalized on the basis of thermal fragmentation to the reactive intermediate (A). This highly electrophilic compound could either react with sulfur nucleophiles to give the observed products arising from transfer, which must compete with the addition of N-benzyl morpholine arising from fragmentation. The latter addition would regenerate the transfer reagent (12). Soft nucleophiles like $Ar\bar{S}$, $ArCH{=}N{-}\bar{O}$, effectively capture the reactive intermediate (A), leading to transfer products. The harder nucleophiles require a much more electrophilic system such as (B) for reactivity. The dimeric products would readily arise from Mannich bases arising from acceptance of (B) (CHART-C- XIX)[45].

Although formamido methyl transfer to sulfur has played a very important role in synthetic organic chemistry, particularly in the preparation of methyl thioisocyanides, the mechanism of this process has not hitherto been subjected to scrutiny. Although further experiments may be needed to convincingly choose from alternate pathways available, the present work has raised aspects of reaction mechanisms which are novel, and which are able to rationally explain the formation of all products.

A suspension of (12) in O-dichlorobenzene or reflux with tetracyclone did not give products that can be traced to a cyclo

adduct. On the other hand, the only product whos structure could be established was dihydrotetracyclone (19), which was obtained in surprisingly good yields (63%). The formation of (19) is rationalized on the basis of a redox reaction involving the iodide counter anion and tetracyclone. The needed hydrogen to form (19) is provided by the electrophilic transfer reagent (12). That such a pathway is feasible, has been demonstrated by heating to 150°C for 8 hr, a suspension of NaI in diphenyl methane, to which tetracyclone was added. The presence of dihydrotetracyclone in this reaction was clearly established (TLC) (CHART-C-XIX).

---

(19): mp: 160°C.

IR: ν_{max}(KBr) cm^{-1}: 3040, 3020, 1690, 1490.

NMR: $\delta(CDCl_3)$: 3.75 (d, J= 7Hz, 1H, benzylic proton), 4.55(d,J = 7Hz, 1H, allylic proton), 7.1-7.4 (m, 20H, aromatic).

ms: m/z : 386 (M^+).

The afficacy of (11) for the transfer of elements of methyl isocyanide has been established via cycloaddition of the conjugate base, generated with nBuLi at -78°C, to acetonitrile and benzonitrile leading to respectively 4-thionaphthyl-5-methyl imidazole (20) and 4- thionaphthyl 5- phenyl, imidazole (21) in 95% and 64% yields (CHART-C- XXI).

CHART-C-XIX

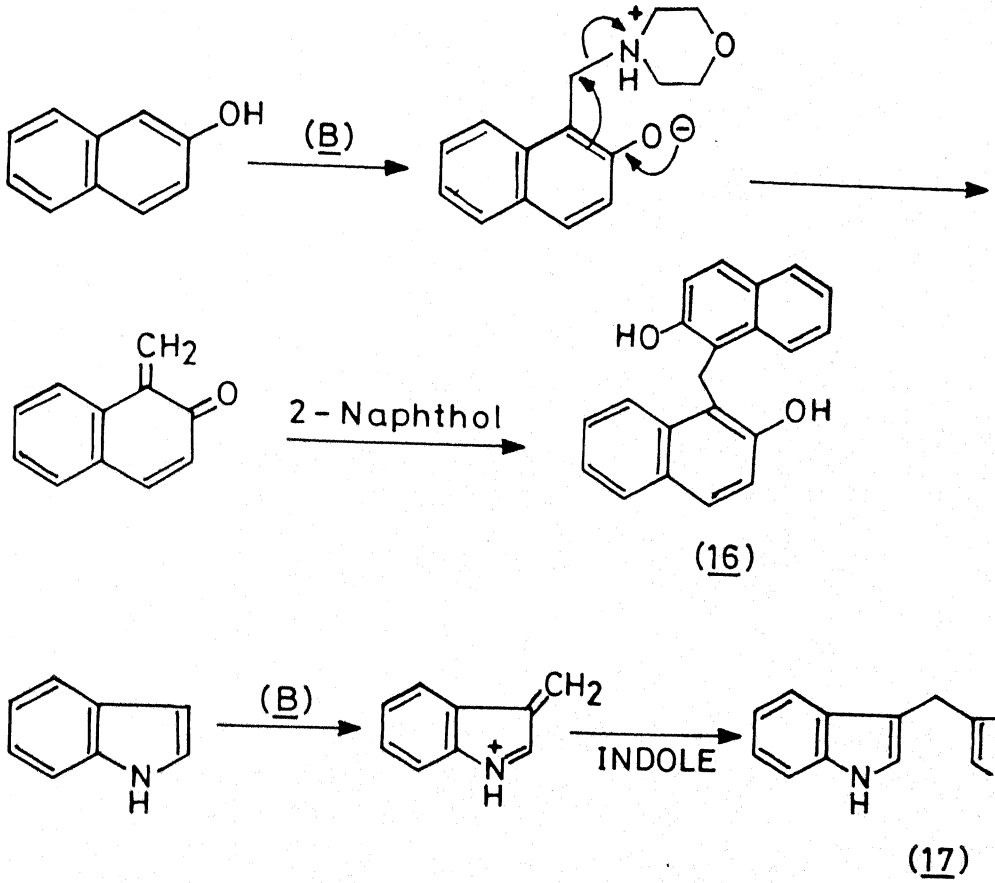
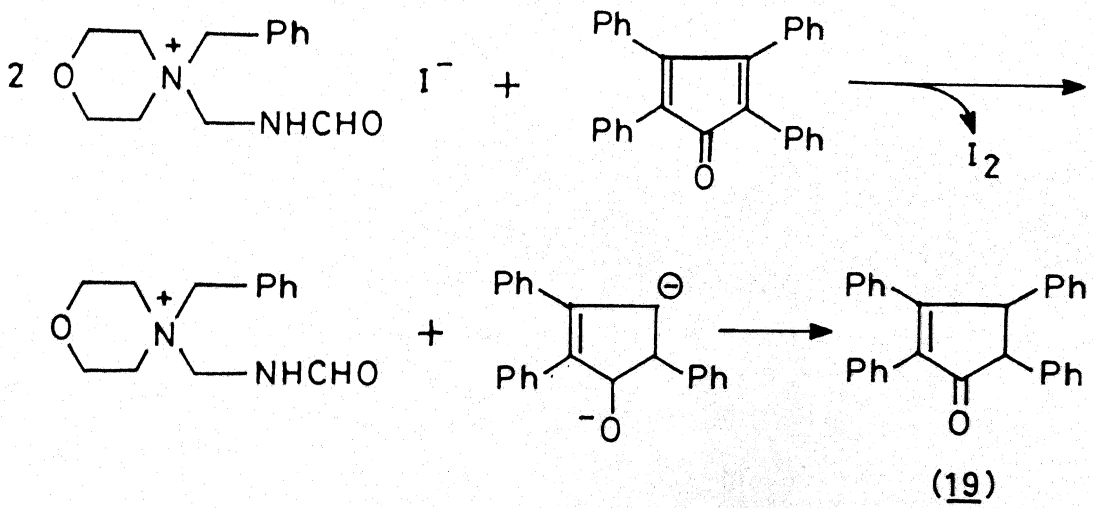


CHART-C-XX



(20): mp. 190° C.

NMR: δ($CDCl_3$+ DMSO-d_6): 2.25 (s, 3H, im-CH_3), 7.05-7.95 (m, 9H, aromatic).

ms: m/z: 240 (M^+).

---

(21) : mp. 223° C.

NMR: δ($CDCl_3$+DMSO-d_6): 7.2-8.05 (m, aromatic).

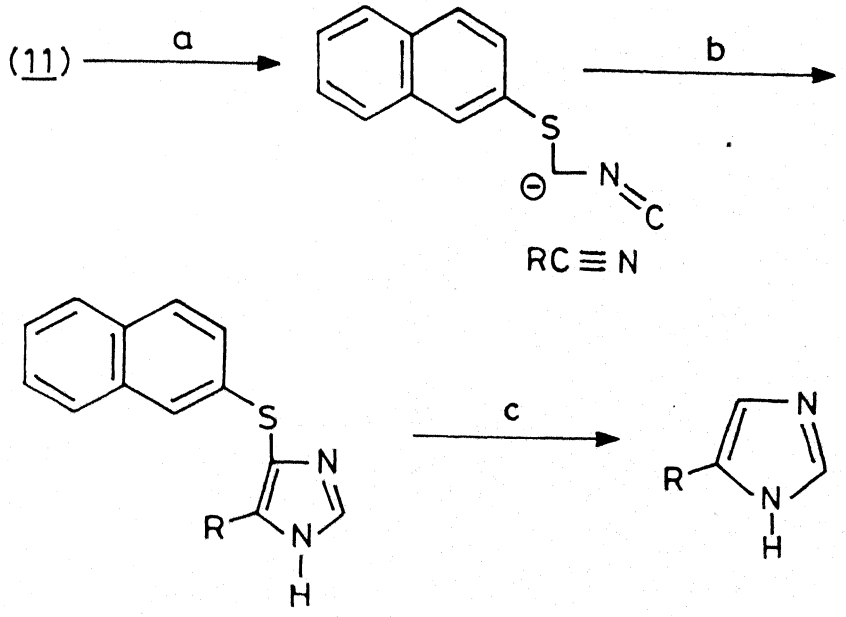
ms: m/z : 302 (M^+).

The 4(5) thionaphthyl imidazoles represented by (20) and (21), are themselves compounds of interest. Apart from the fact that the sulfur ligand will modulate the amphoteric nature of the imidazole, this ligand enables to distinguish the two nitrogens. Steric factors here would dictate alkylations or acylation at the site away from the proximate nitrogen. Desulfurization of such products would provide a very facile route to 1-substituted-5 liganded imidazoles. Inspite of continuing interest in the chemistry of imidazoles, procedures for the preparation of 1-protected-5- substituted imidazoles are quite scarce[46].

Although mention was made in the literature to the effect that compounds of the type (20) and (21) could be desulfurized to their corresponding imidazoles, the specific procedure was not available[47]. Initial experiments to effect this reaction by standard procedures yielded no desirable results. Eventually it was found that the transformation could be effected in excellent yields by treatment with freshly prepared W-6 Raney Ni at rt, for 5hr. Thus compounds (20) and (21) were transformed to respectively 4(5) methylimidazole (22), (76%) and 4(5) phenyl imidazole (23), (56%) (CHART-C- XXI). The melting points and NMR data of (22) and (23) were identical to that reported in literature[48].

Having developed a very satisfactory method for the R-CN to R-imidazole change, endeavours were made to translate this to the peptide domain and towards this, the reaction of Z-Ala-(β-Cyano)-OMe with the conjugate base of (11) was extensively studied. As shown in CHART-C- XXII the expected adduct from this reaction (24) on desulfurization would lead to Z-His-OMe. In several of the reactions, compound that exhibited NMR expected of the adduct (24) was isolated by preparative TLC. However, the amounts were very small. Surprisingly, the major part was an intractable acidic mixture. Blank experiments showed that whilst (10) is sensitive to the conditions used for the addition, the corresponding carboxylic acid was largely not affected. However, when such cycloadditions were carried out with Z-Ala-β Cyano-OH, no product corresponding to the expected adduct was encountered.

CHART-C-XXI

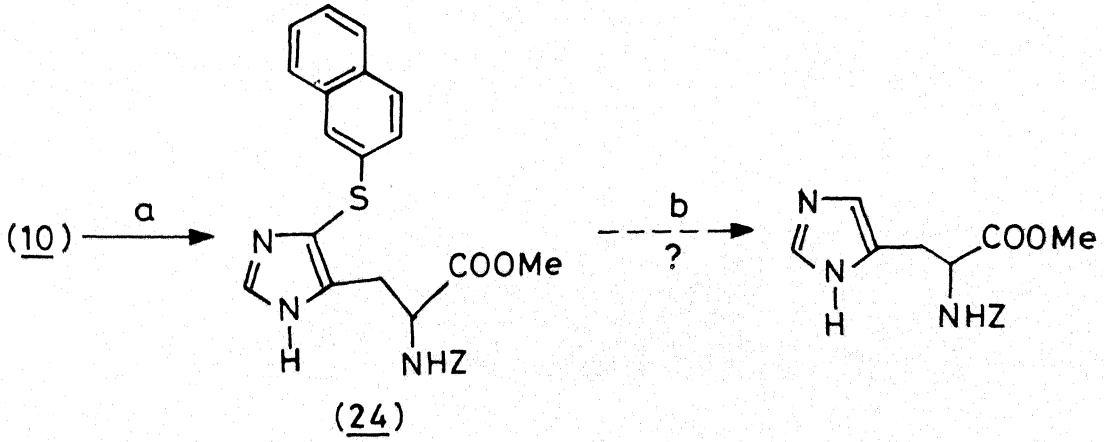


(20): R = Me
(21): = Ph

(22): R = Me
(23) = Ph

a : nBuLi, −78°C
b : RCN, THF
c : W-6, Raney Ni

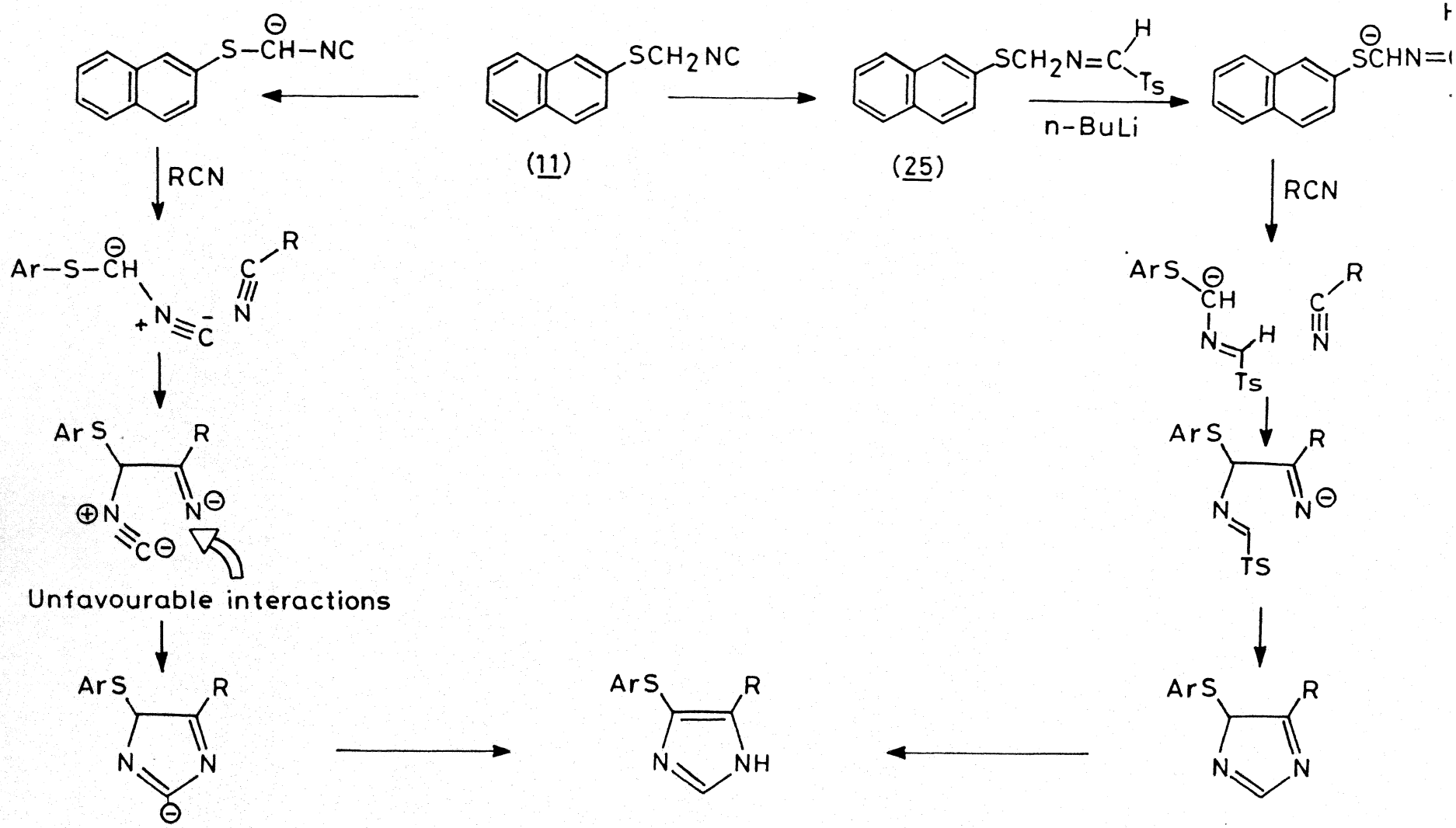
CHART-C-XXII



(24)

a : (11), nBuLi, −78°C
b : W-6, Raney Ni

CHART-C-XXIII



The failure to effect the cycloaddition to Z(α) β-cyano alanine methyl ester (10), is most probably due to problems associated with the generation of conjugate base of the (11) and its propencity to effect undesirable side reactions. It was felt that these problems could be solved via increasing the acidity of (11) and, at the same time, increasing the propencity for the desired cycloaddition. This was sought to be accomplished via "p-$CH_3C_6H_4SO_2H$ adduct of (25) (CHART-C- XXIII). Compound (25), would not only be more acidic compared to (11), but also, as shown in CHART- C- XXIII, the unfavourable charge-charge interaction in the transition state involving the conjugate base of (11), would be absent in cycloaddition mediated by conjugate base of (25). Preliminary experiments to prepare (25) by addition to (11) did not succeed.

STUDIES ON THE CHEMICAL SIMULATIONS OF POST TRANSLATIONAL LYSINE HYDROXYLATIONS

The genesis of investigations thus far described, had a bearing on the selection of coded amino acids. Equally interesting are post translational processes involving highly selective side chain alternation of proteins. These process must also have evolved over long time spans to optimize the cell requirements. This aspect finds apt illustration in the post translational modifications of the proline and lysine residues of the structural protein collagen. The most noteworthy feature of this operation is that an inactive CH_2 function becomes the target for selective hydroxylation. In the present work, preliminary studies, pertaining to the lysine--→δ - hydroxy lysine modification have been done.

Nascent collagen consists of approximately 4% of lysine. This residue progressively gets hydroxylated. Thus there is a direct correlation of the amount of hydroxylysine to the age of the species. The presence of δ --hydroxylysine reflects one facet brought about with agae, namely, enhanced collagen cross linking and shrinkage. The mechanism of the lysine --→δ-hydroxylysine change is mediated by an enzyme carrying an Fe^{+2} prosthetic group, and requires α- ketoglutaric acid and molecular oxygen. The primary process is the transformation of -ketoglutaric acid to mono per acids of glutaric acid. The

pathway with which this key compound arises, is presented in CHART-C-XXIV[48].

The mechanism by which the pre acid, either free or enzyme bound, brings about selective hydroxylation of substrate, is not established. In the present work as a working model, this reaction was envisaged via pathways illustrated in CHART-C-XXV. Thus the desired hydroxylation could take place via sequence, schiff base intermediate, covalent bond transposition as that takes place in the Barton reaction, followed by hydrolysis.

This notion coupled with the demonstrated possibilities for attachment of pendant groups in proteins, (vide supra), made it attractive to use a quinazoline ring attached to the ω -amino group of the lysine (Q-Lys) as an appropriate model, to bring about the selective hydroxylation. At the outset, it was envisaged that Q-Lys could bring about the desired change via selective abstraction of the δ-hydrozen via a six membered transition state as illustrated in CHART-C-XXVI. The radical thus generated would lead to hydroxylation and the pendant group can be subsequently hydrolytically delinked, to form the δ-hydroxylysine site change.

Lysine was converted to the copper complex, which on reaction with 4-chloroquinazoline, led to the attachment of the pendant moiety leading to the Q-Lys-Cu complex (26) in 82% yeilds. Compound (26) was then transformed to N-benzyloxycarbonyl ω-amino quinazoline lysine (27), (50%) as well

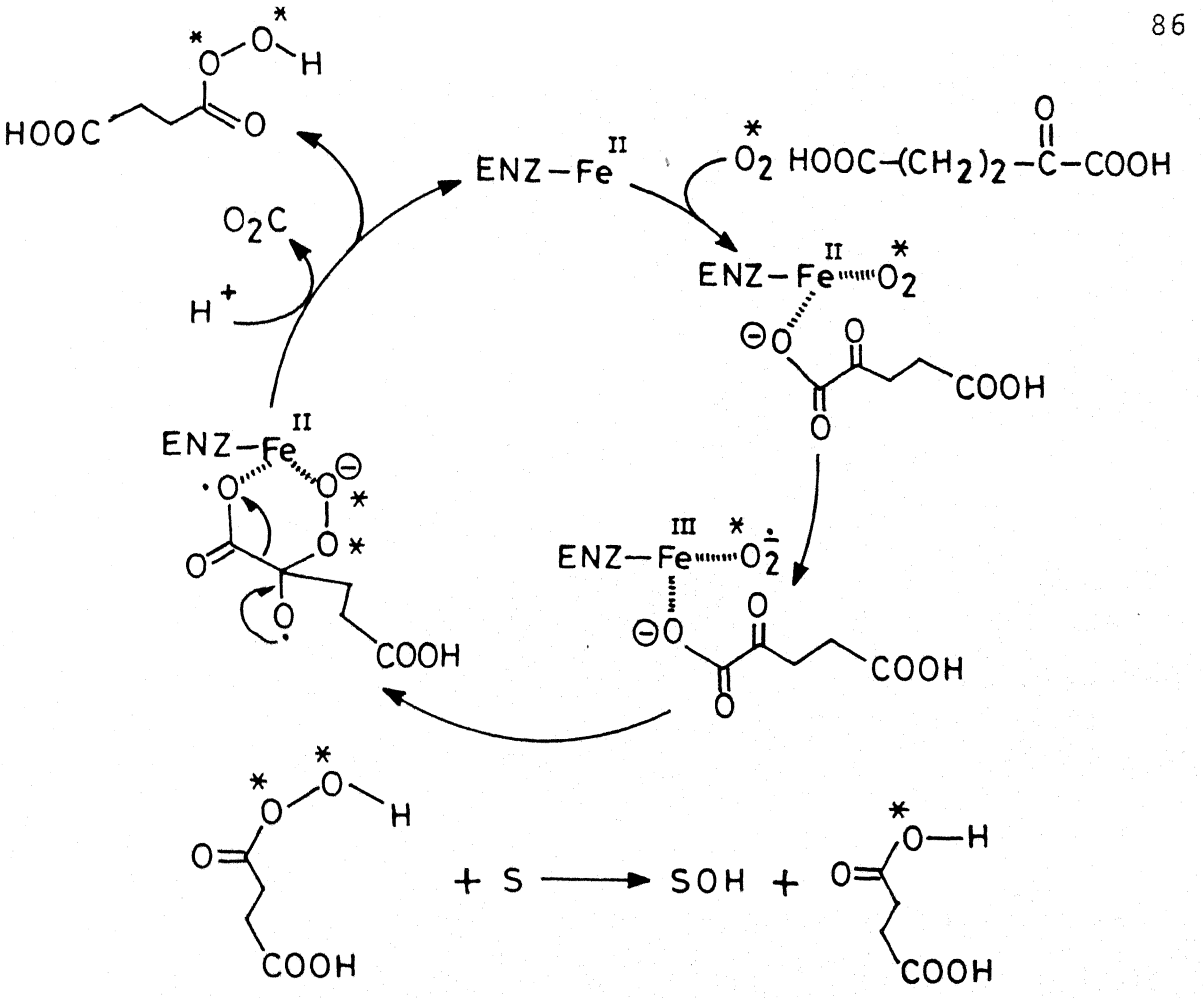
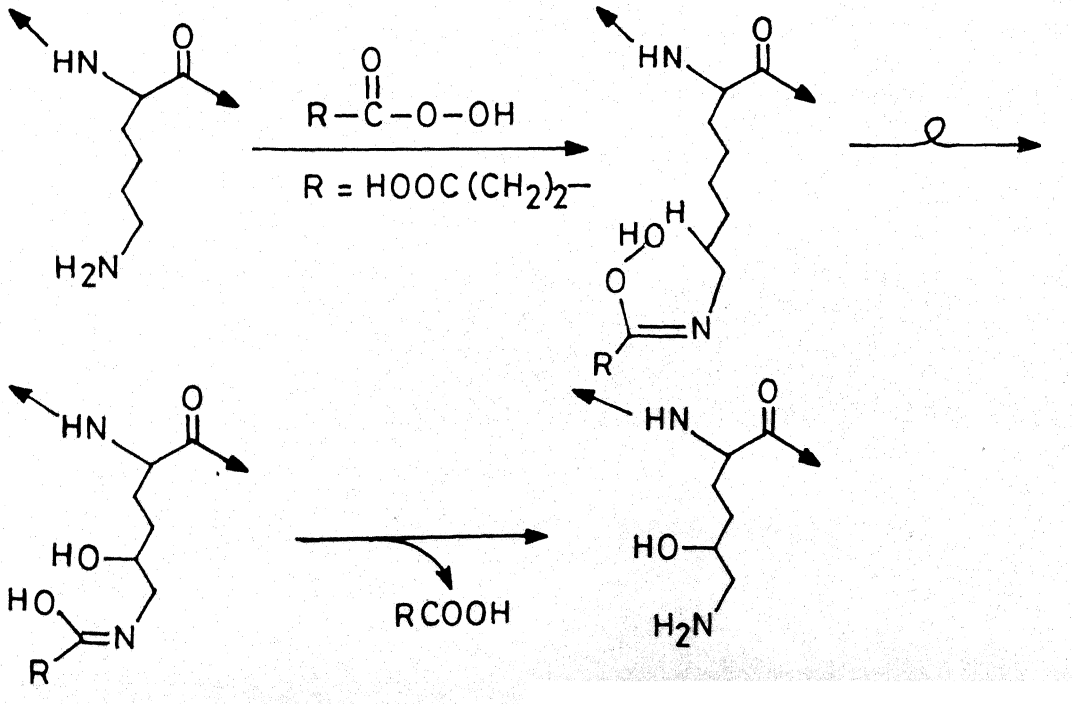

HOOC
ENZ–Fe II
O2 HOOC–(CH2)2–C–COOH
O2C
H+
ENZ–Fe II O2
COOH
ENZ–Fe II
COOH
ENZ–Fe III O2
COOH
+ S
SOH +
COOH
COOH

CHART-C-XXV


HN
H2N
R–C–O–OH
R = HOOC(CH2)2–
HO
N
R
RCOOH
H2N

as to N-benzoyl - ω -amino quinazoline lysine(28), (45%) (CHART-C-XXVII).

---

(26): mp. 210°C (d).

IR: ν_{max} (KBr) cm^{-1}: 3300, 1690, 1625, 1610.

---

(27) : mp. 165°C.

IR: ν_{max} (KBr) cm^{-1}: 3300, 1740, 1650, 1610, 1550 (sh).

---

(28): mp. 185°C.

IR: ν_{max}(KBr) cm^{-1}: 3445, 3300, (-NH), 1750, 1650, 1550 (amide).

NMR: δ ($CDCl_3$+DMSO-d_6): 1.25-2.15 (m, 6H, $-(CH_2)_3$), 3.9 (m, 2H, $-NH-CH_2$), 7.3-8.7 (m, 10H, aromatic).

ms: m/z: 378 (M^+).

The hydroxylation studies were to be initiated on fully protected esters arising from simple esterification of the carboxylic function in either (27) or (28). In the event, this could not be realized. Instead, the reaction of (27) with diazomethane followed by chromatography gave the N-methylated compound (29) (CHART-C-XXVIII).

---

(29): mp. 140°C.

IR: ν_{max} (KBr) cm^{-1}: 3420, 3400 (-NH), 1740 (ester), 1720, 1660, 1560 (amide).

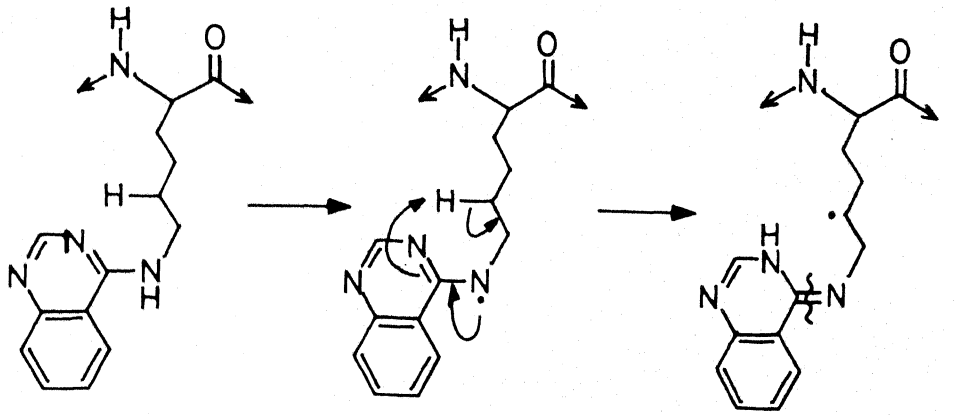
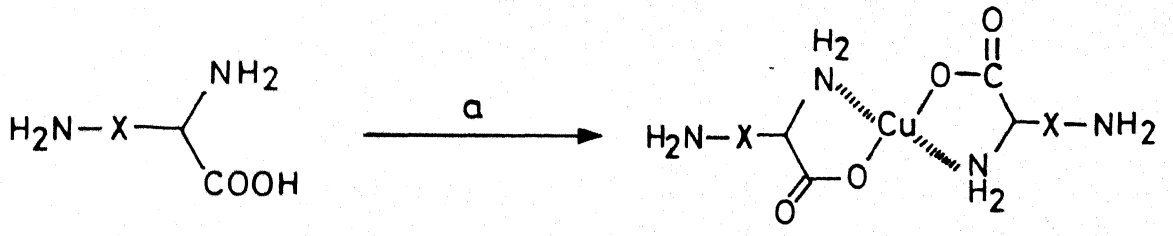
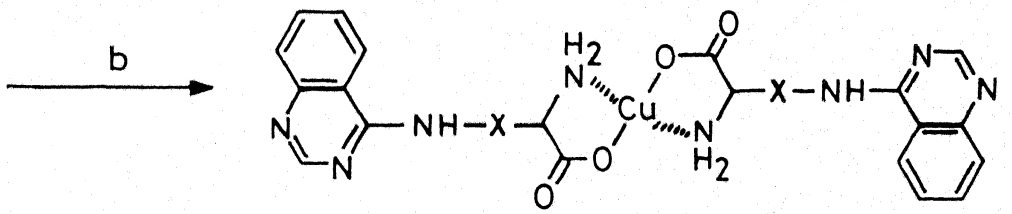


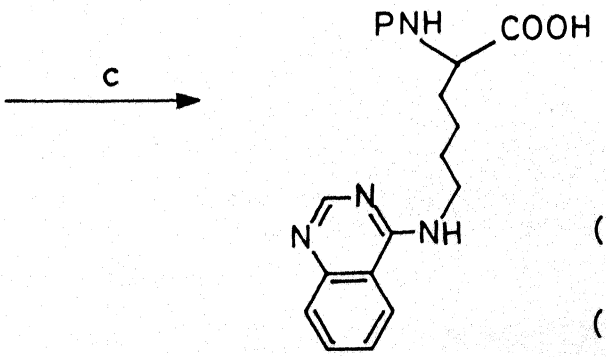
CHART - C - XXVII



X = $(-CH_2)_4$

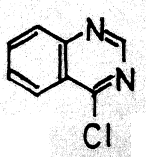


(26)



(27) : P = $-C(=O)-OCH_2Ph$

(28) : P = $-C(=O)-Ph$

a : $Cu(OH)_2$, $CuCO_3$ b:  c : H_2S, H^+, PCl

ms: m/z : 437 (M^+).

In an alternate strategy, the lysine copper complex was treated with benzyloxycarbonyl chloride, the copper complex decomposed, the α-amino function protected as benzoyl, the acid function esterified to give α-benzoyl, ω-benzyloxycarbonyl lysine methyl ester (30). The benzyloxycarbonyl group was removed by hydrogenolysis and the Bz-Lys-OMe thus obtained was treated with 4-chloroquinazoline. Surprisingly, in sharp contrast to the reactivity of lysine copper complex towards chloroquinazoline, attempted reactions of Bz-Lys-OMe, with the same compound did not succeed.

---

(30): IR: ν_{max} (thin film) cm^{-1}: 3320 (-NH), 2970, 1760 (ester), 1710, 1650, 1550 (amide).

NMR: δ($CDCl_3$): 0.9-2.15 (m, 6H,(CH_2)$_3$),3.35 (m,2H,-NH-CH_2-), 3.65 (s, 3H, -O-CH_3), 5.0 (s, 2H, O-CH_2-Ph),7.1-7.85 (m, 10H, aromatic).

The formation of (29) would imply that the pKa of the carboxyl group and the pendant moiety are comparable. Fortunately such a problem would not be present under actual peptide environments and therefore the hydroxylation studies can be carried out at the earlier stage itself. Paranthetically, (27) and (29) represent novel systems possesing high reactivity. Consequently, the ease with which the pendant could be attached in a true peptide environment, should provide a facile route to a

variety of modified lysine side chains. For example, in (27), (28) and (29) hydrolytic cleavage of the 2-3 bond of quinazoline, which is an exceptionally facile operation, would lead to novel arginine anologs, where one of the amino groups of this moiety would be distanced by an orthobenzeniod spacer.

In view the difficulties encountered with respect to the preparation of the target molecule required for the functionalization studies, it was considered profitable to perform such studies on the Q-Lys copper complex (26). Amongst the several procedures tried, that of the reaction of (26) with ferric chloride in aq. acetic acid involving oxygenation for 24 hr, followed by removal of copper, benzoylation and esterification gave, surprisingly, in 13% overall yeild, the dibenzoyl lysine methyl ester (31).

---

(31): IR: ν_{max} (thin film) cm^{-1}: 3315 (-NH), 1725, 1630, 1570 (amide).

NMR: δ ($CDCl_3$): 1.4-2.2 (m, 6H, $-(CH_2)_3$), 3.4 (m, 2H, $--NH-CH_2$), 3.75 (s, 3H, $O-CH_3$), 4.75 (m, 1H, tertiary proton), 7.2-7.9 (m, 10H, aromatic).

ms: m/z: 368 (M^+).

Blank experiments showed that the transformation involves the $FeCl_3$/aq.CH_3COOH,O_2 treatment. The genesis of (31) is rationalized in CHART-C-XXIX, and is thought to involve the formation of quinazoline-N-oxide, which, because of its higly enhanced susceptibility to hydrolysis, could be expected to lead

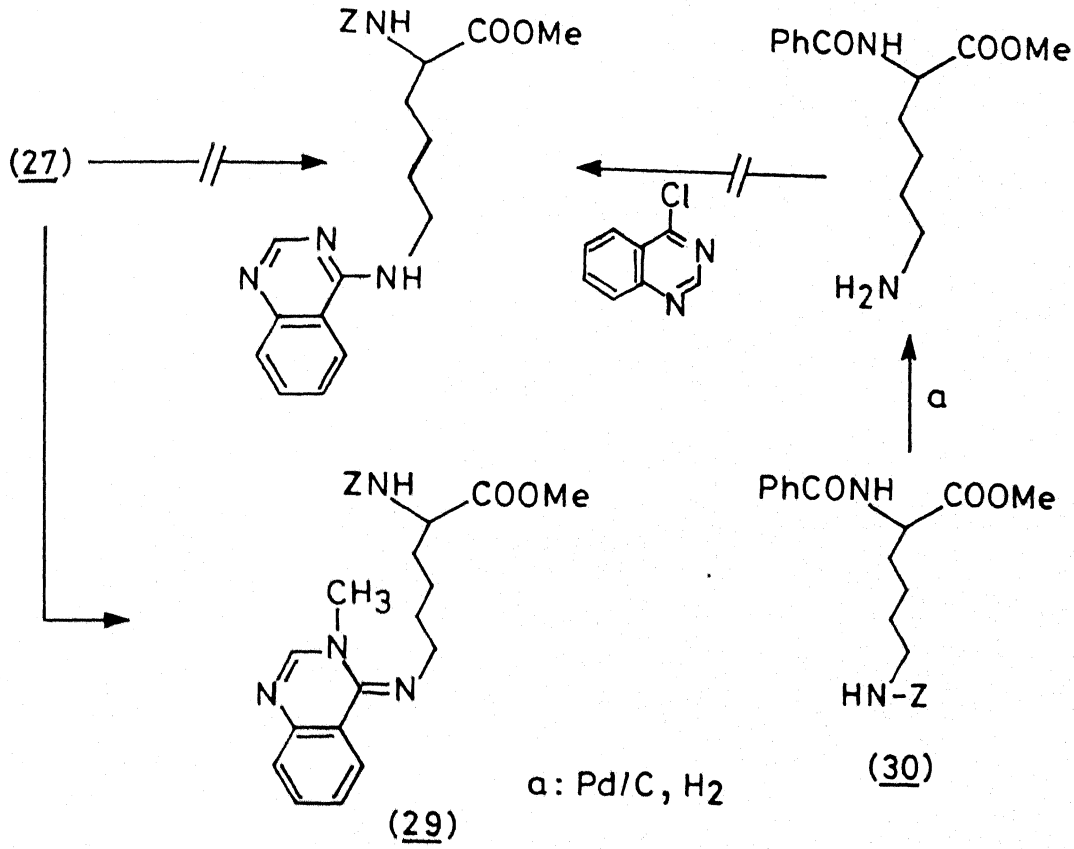
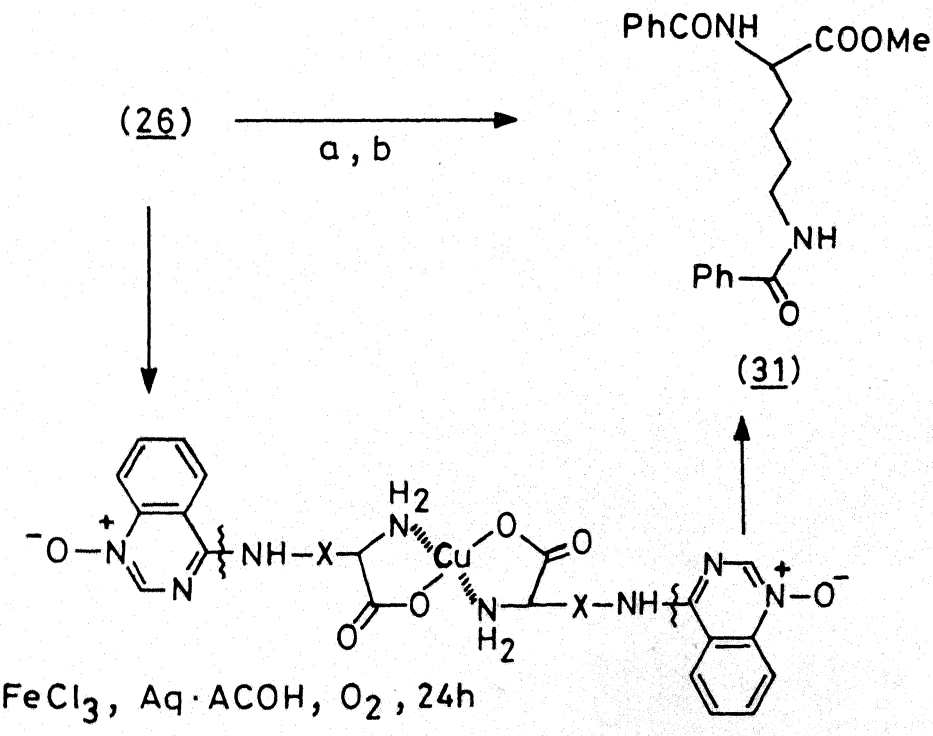

ZNH
COOMe
(27)
N
NH
PhCONH
COOMe
Cl
N
N
H_2N
a
ZNH
COOMe
CH_3
N
N
N
PhCONH
COOMe
HN-Z
(29)
(30)
a: Pd/C, H_2

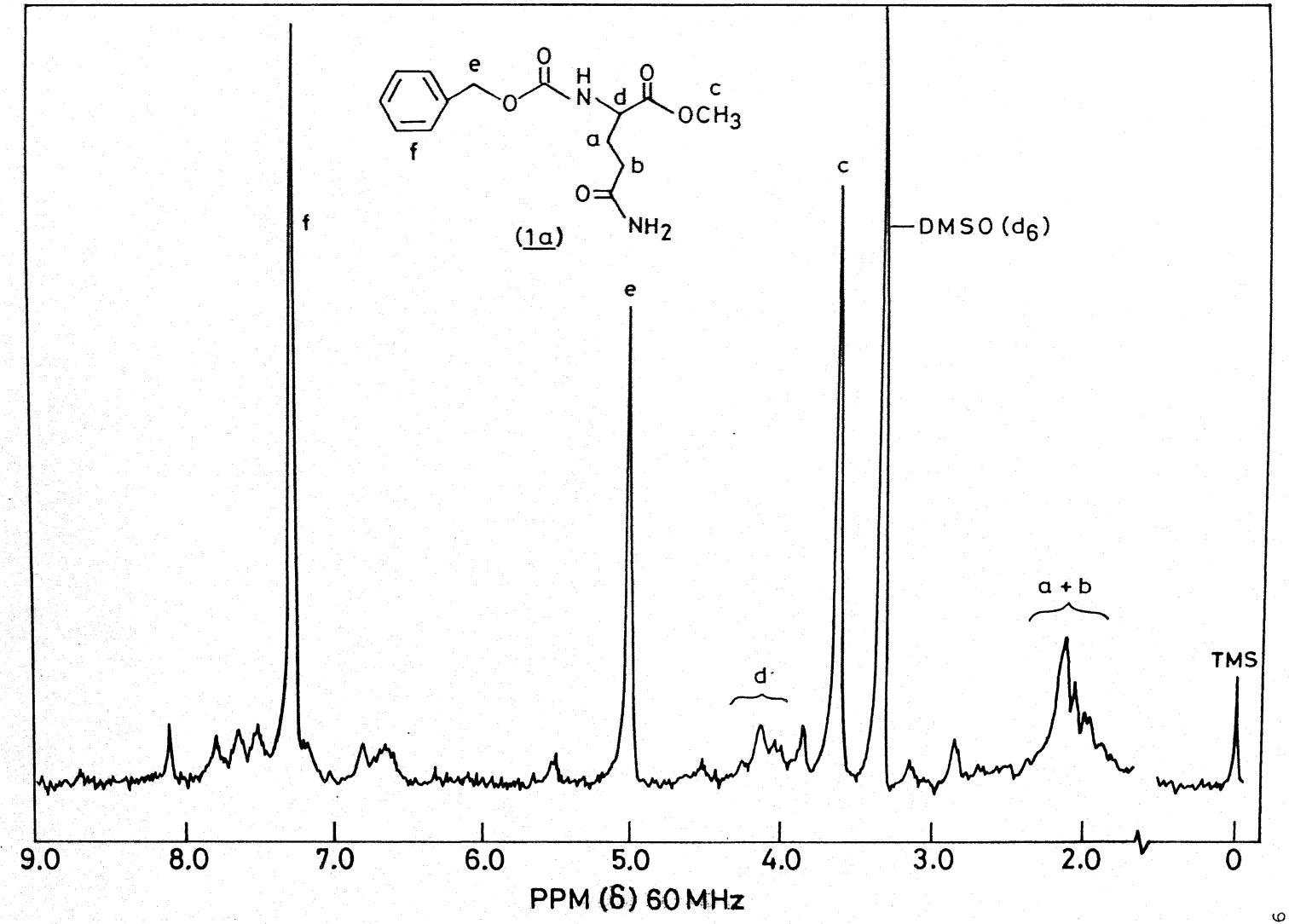
CHART-C-XXIX

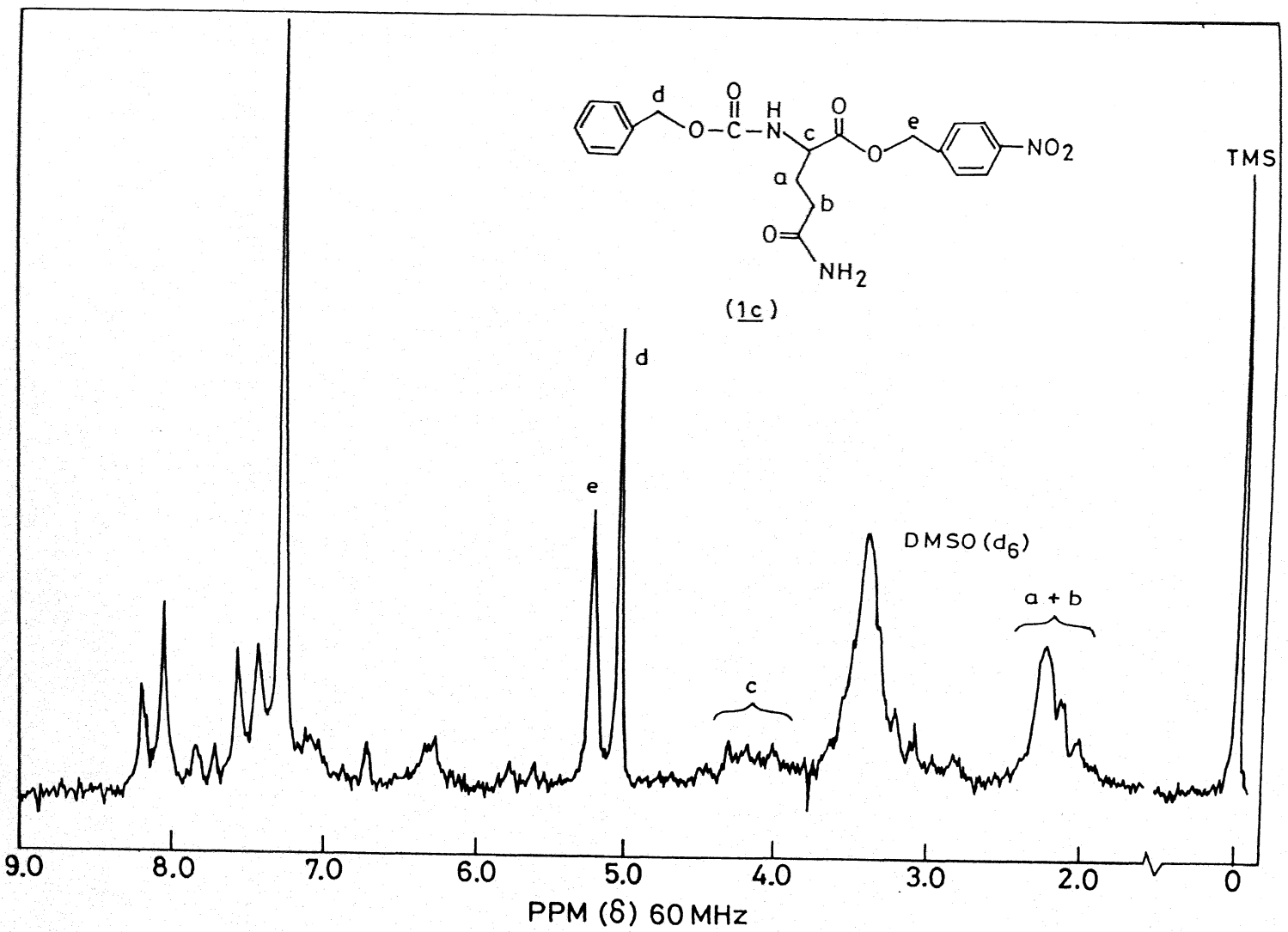

PhCONH
COOMe
(26)
a, b
NH
Ph
O
(31)
$^{-}O-N^{+}$
N
NH-X
H_2
N
Cu
O
O
O
O
N
H_2
X-NH
N
$N^{+}-O^{-}$
a: $FeCl_3$, Aq·ACOH, O_2, 24h
b: H_2S/Bz-Cl, CH_2N_2

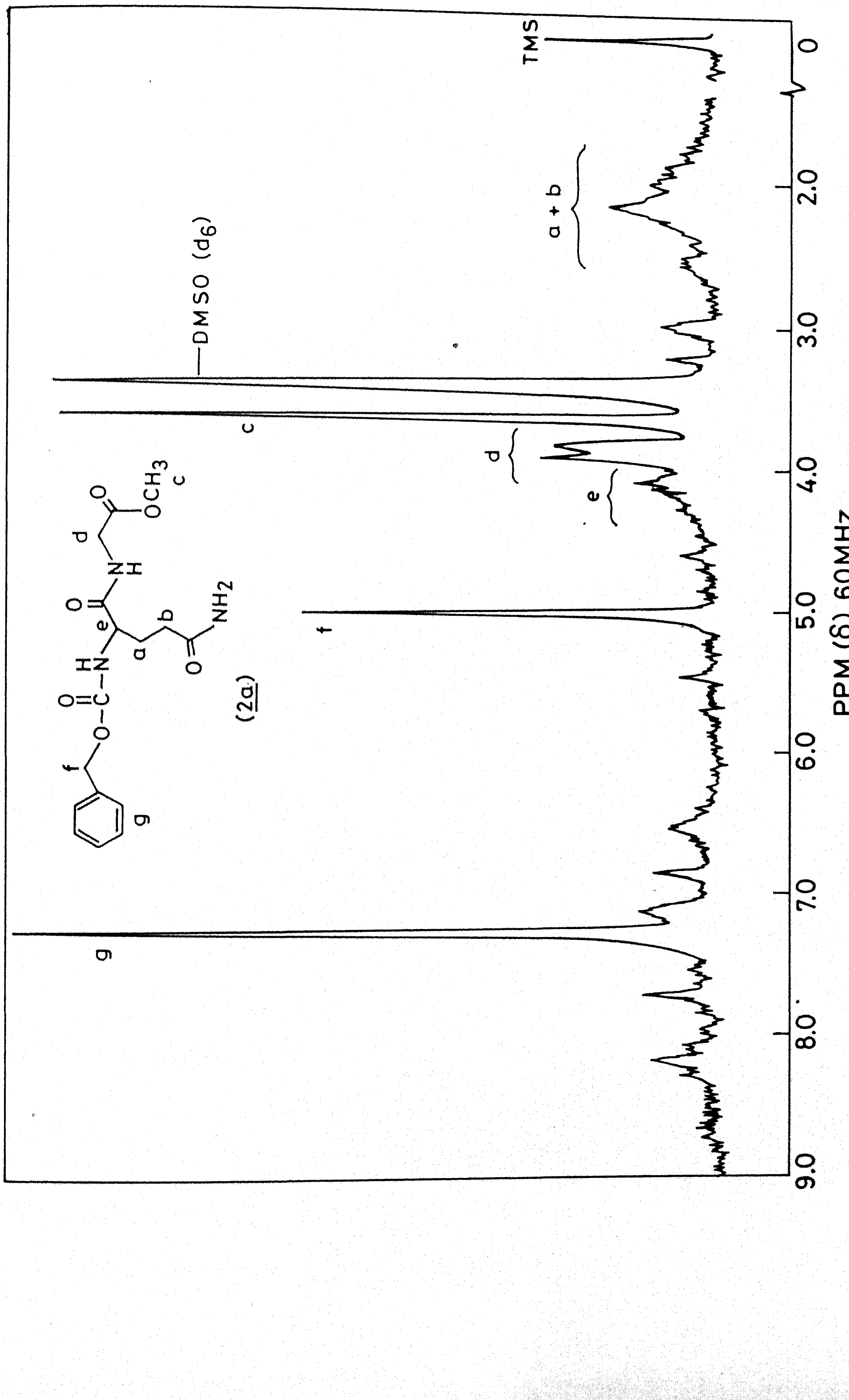
to compound (31).

One of the major objectives pertaining to the chemical simulation of lysine δ-hydroxylation, namely the attachment of an appropriate pendant, has been achieved. However, the use of this moiety to bring about the desired reaction remains largely to be explored.

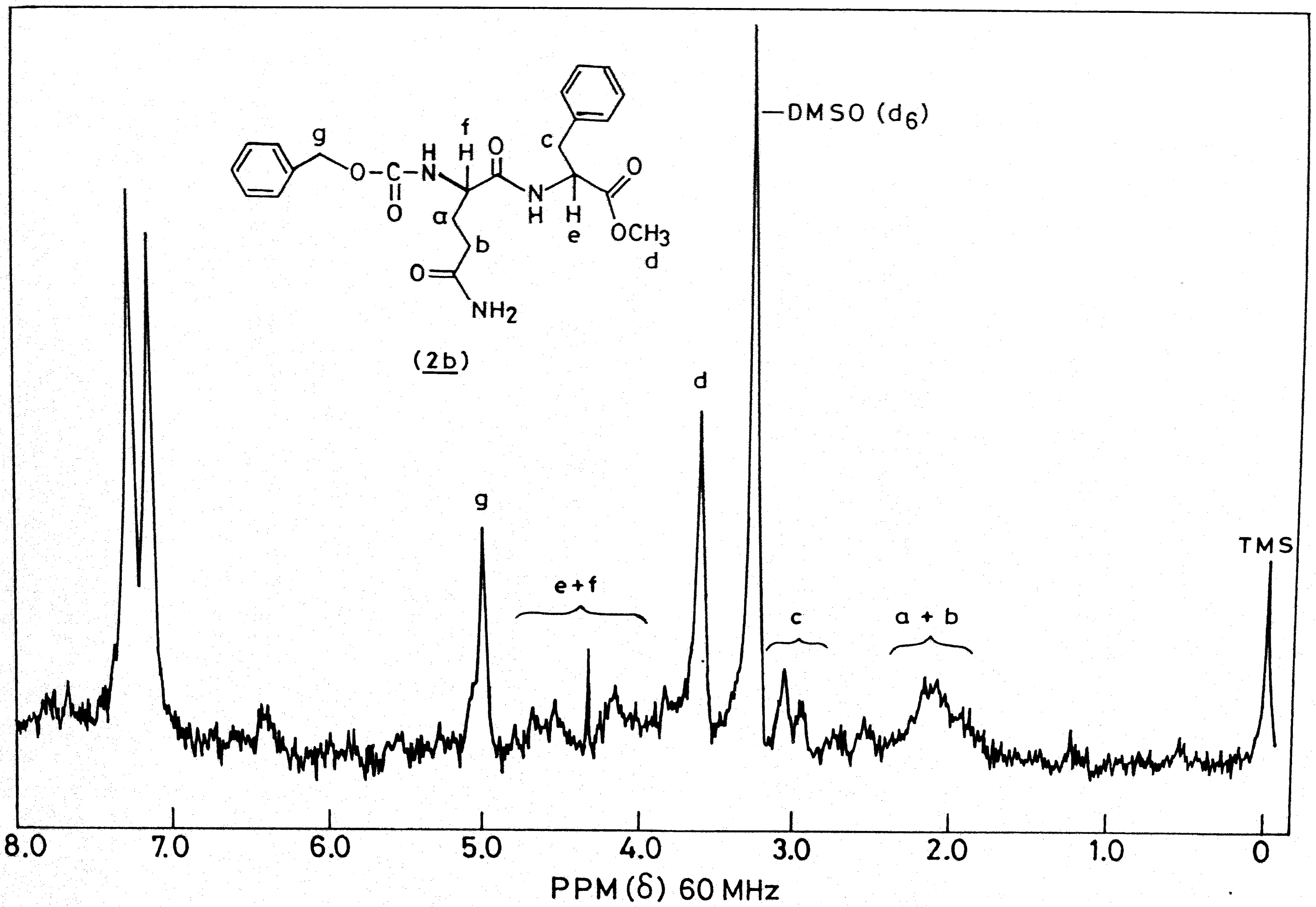
The genesis of lysine as a component of the coded system, the creation of an imidazole ring from glutamine side chain, and the chemical simulation of the post translational hydroxylation of lysine, constitute the line of investigations persued in the present work. In some cases the desired objectives have been attained, a number of unusual facets pertaining to the chemistry of coded amino acids side chains have been encountered, and, above all, the work has been able to provide a number of interesting pathways for future fruitful investigation.

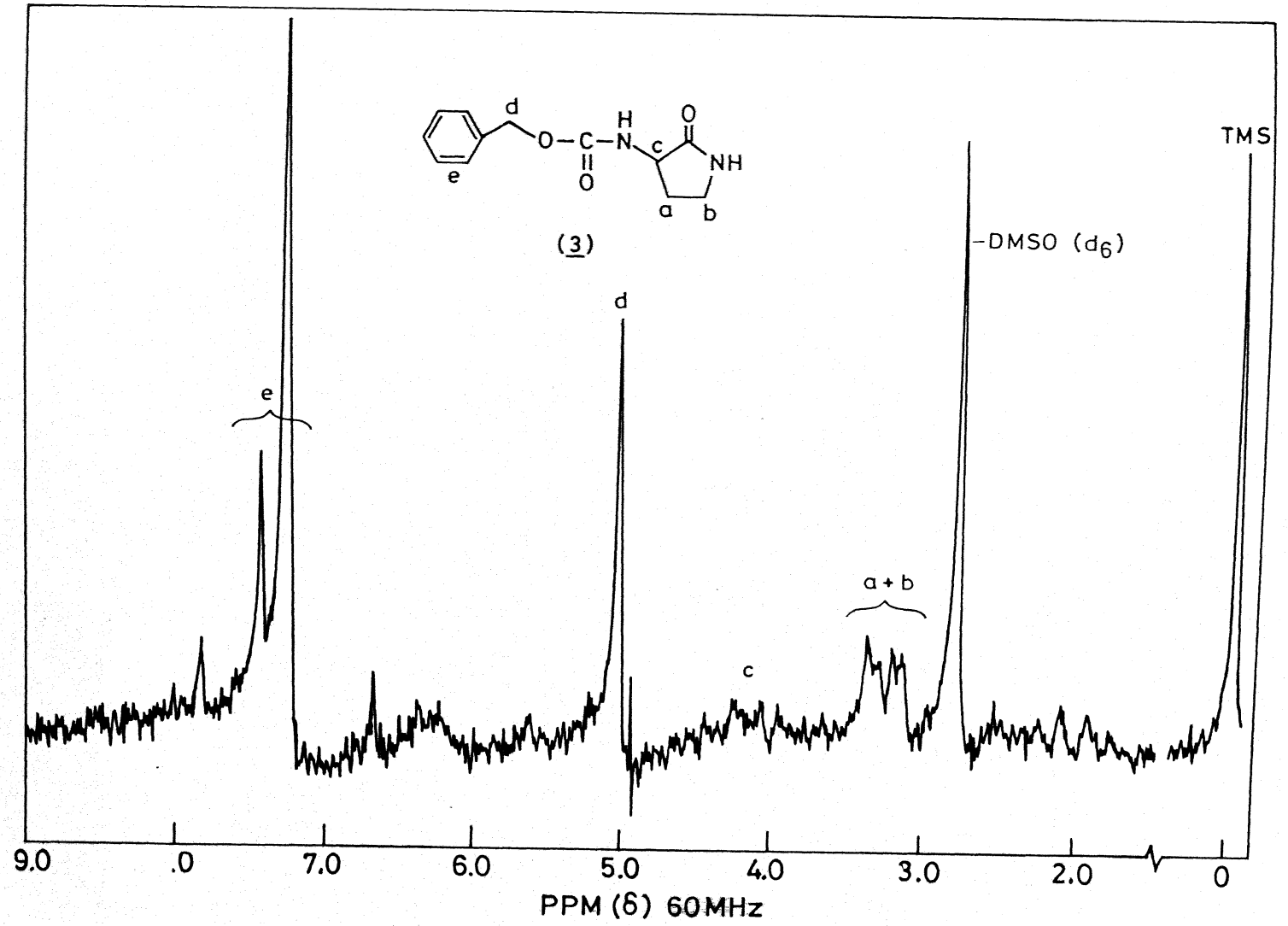

f
e
d
c
DMSO (d6)
a + b
TMS
(1a)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
0
PPM (δ) 60 MHz

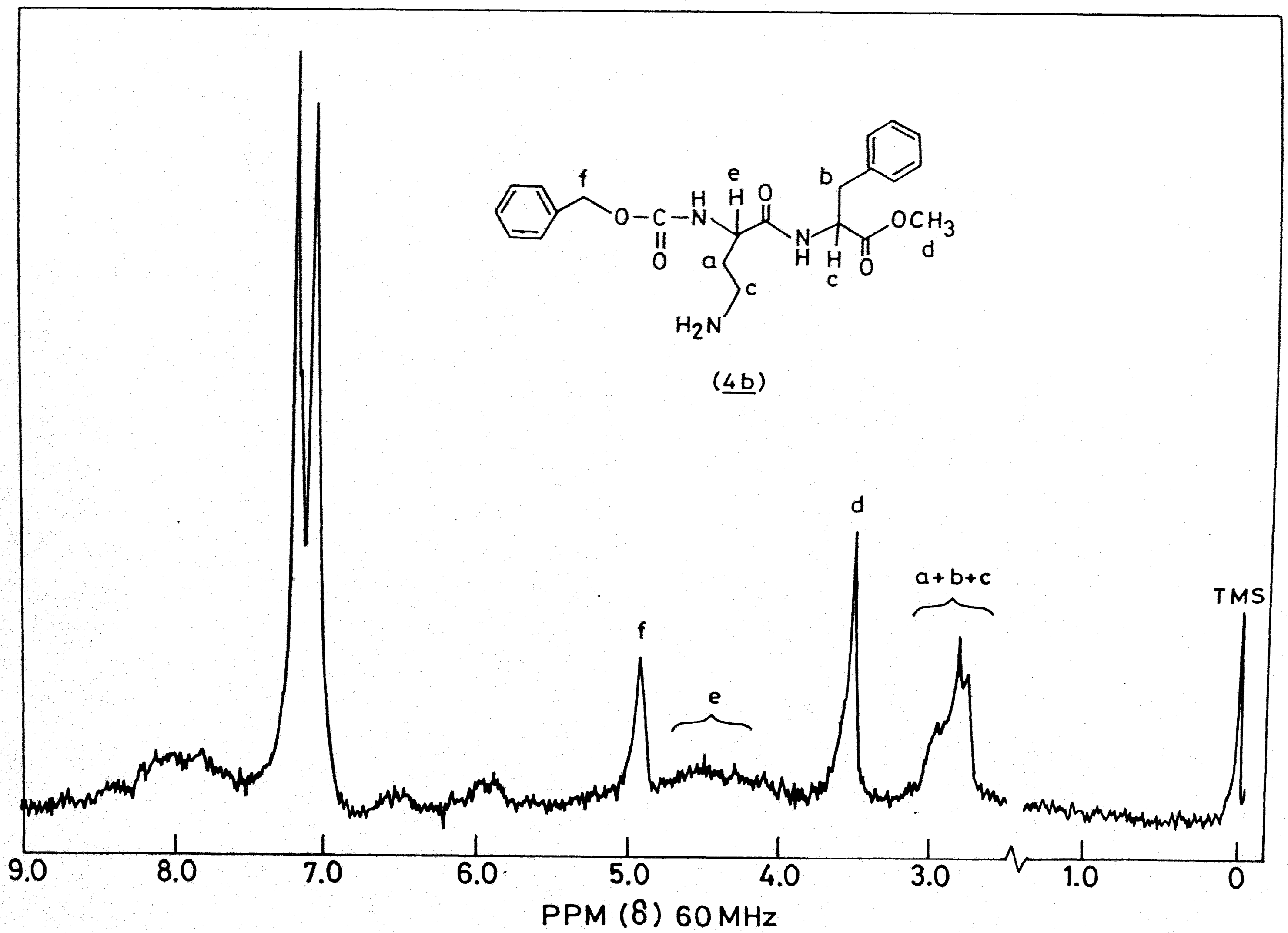

d
O
H
O
e
O-C-N
c
O
NO2
a
b
O
NH2
(1c)
TMS
d
e
DMSO (d6)
a + b
c
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
0
PPM (δ) 60 MHz

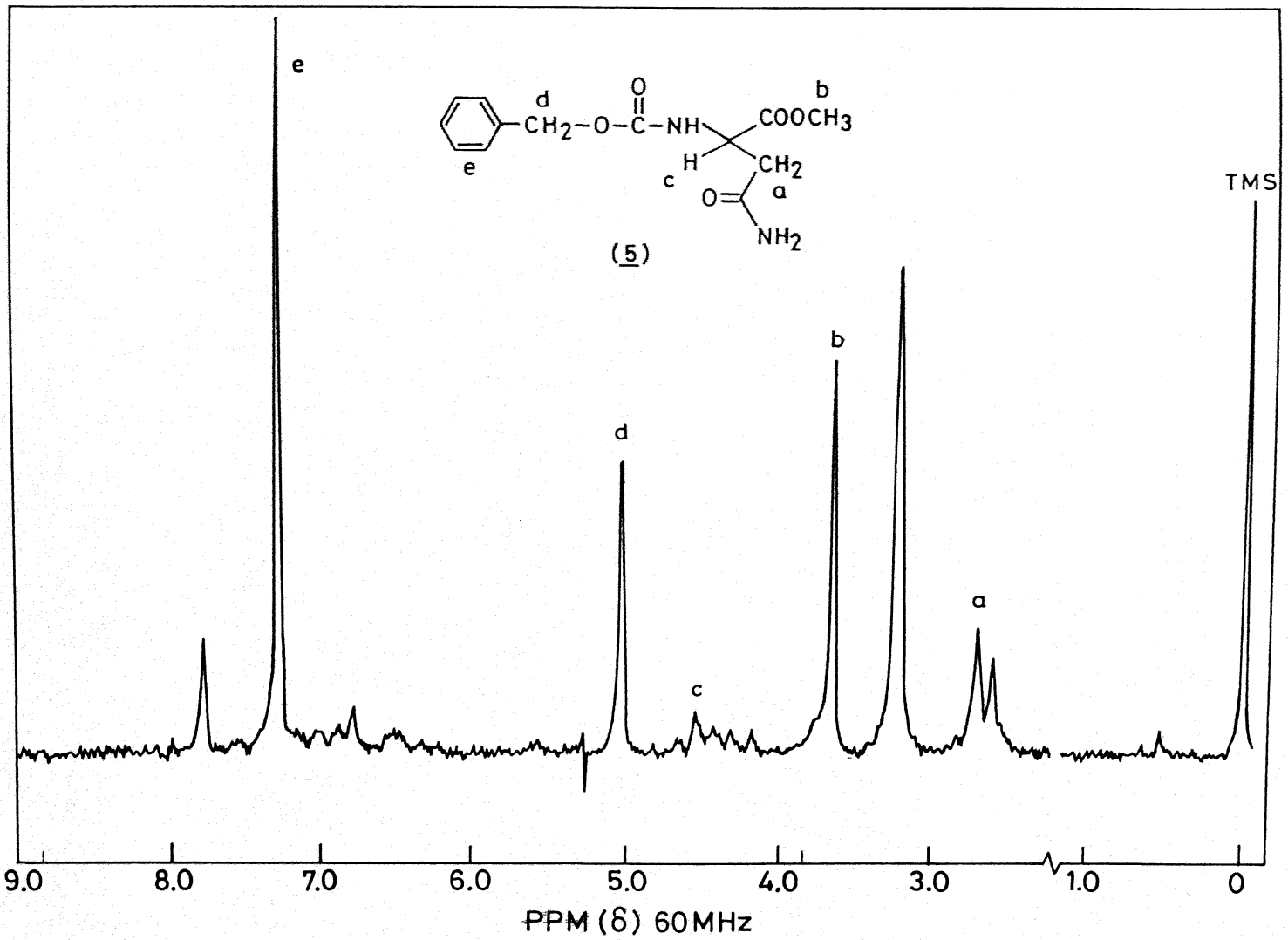


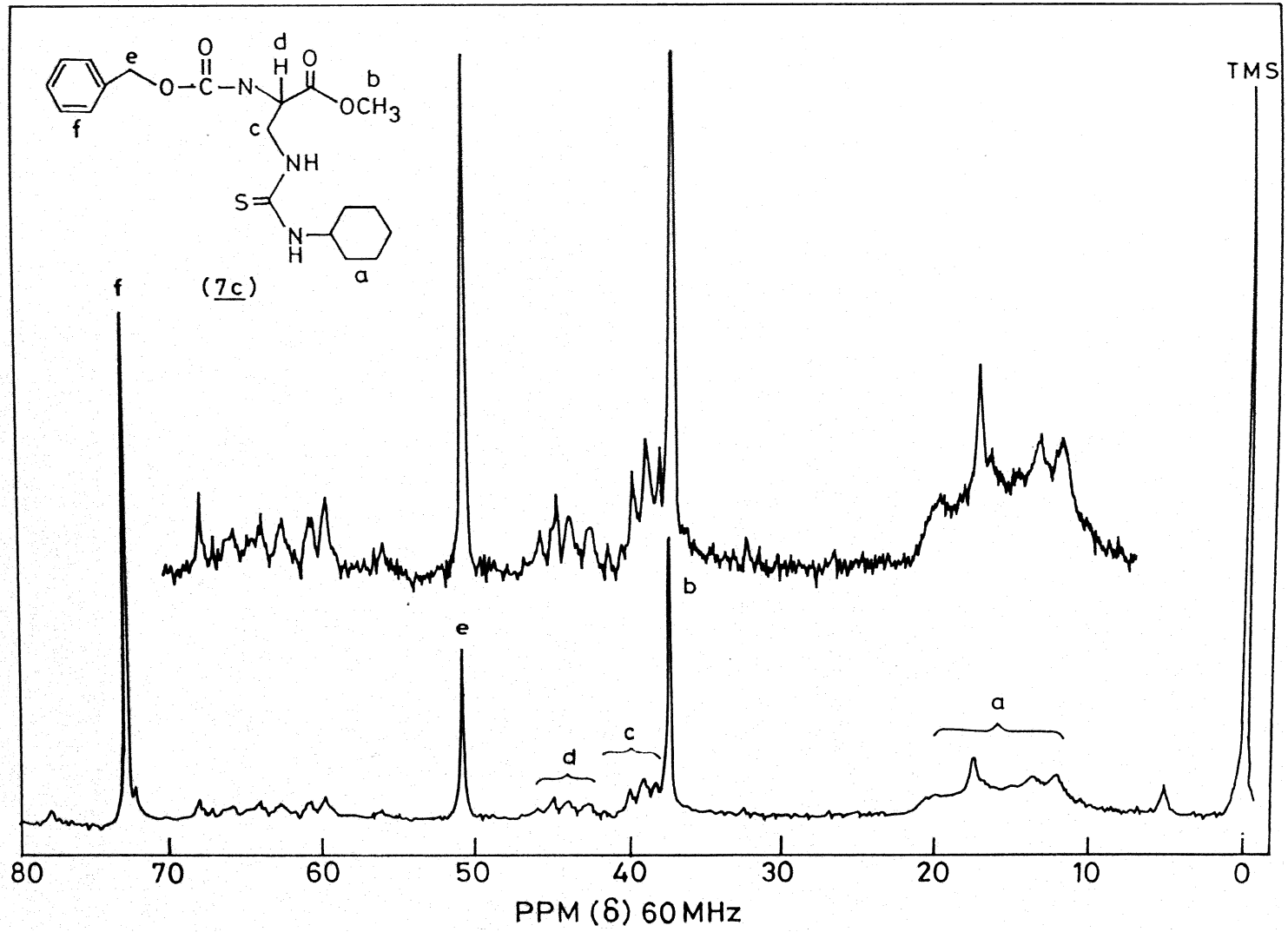
TMS
a + b
DMSO (d6)
c
d
e
f
g
(2a)
0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
PPM (δ) 60MHz

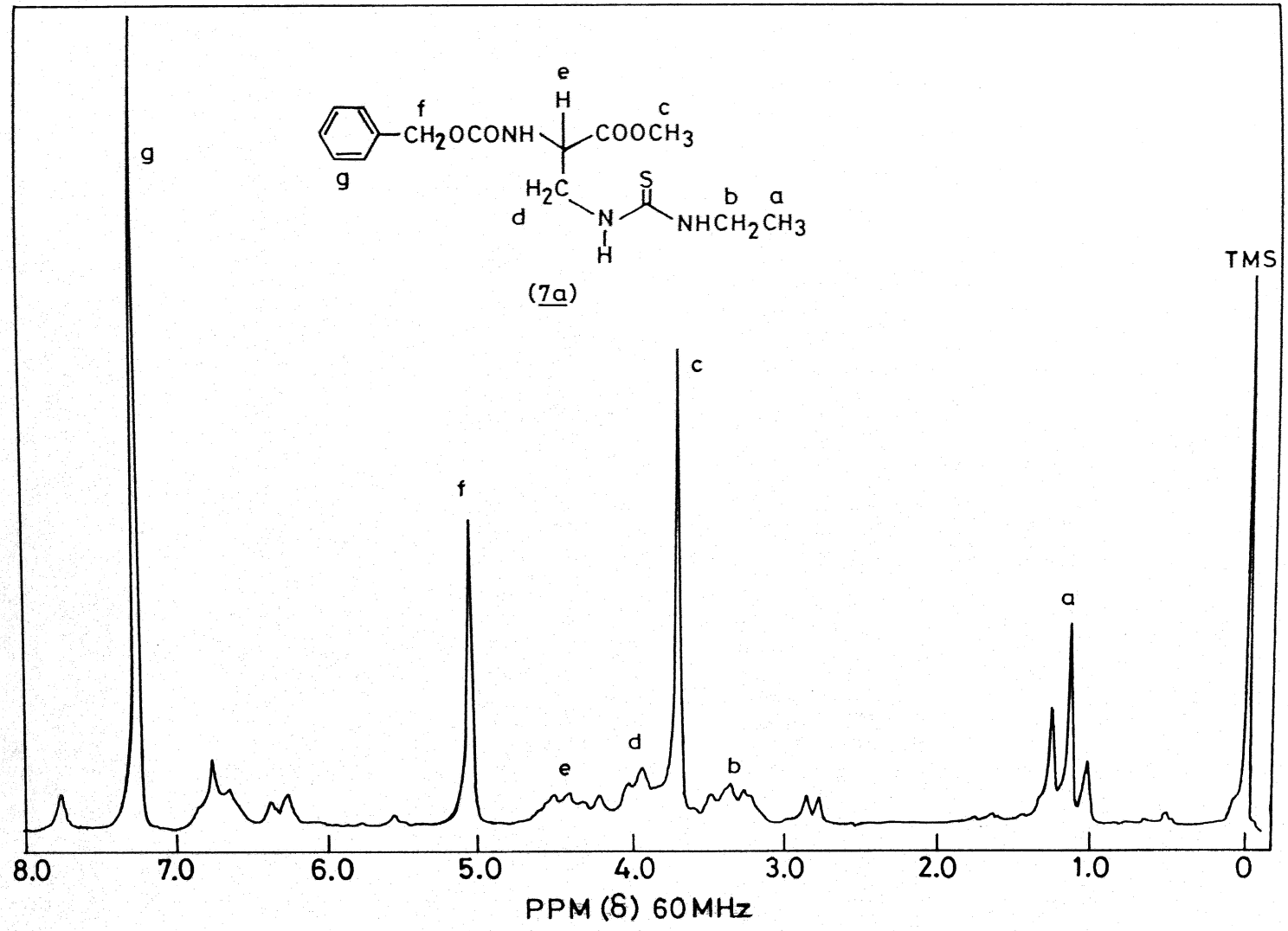

g
f
H
H
O
c
O
O-C-N
N
O
a
H
H
b
e
OCH3
d
O
NH2
(2b)
DMSO (d6)
d
g
TMS
e+f
c
a + b
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ) 60 MHz


d
e
O
C
H
N
c
O
NH
a
b
(3)
TMS
DMSO (d6)
d
e
a + b
c
9.0
.0
7.0
6.0
5.0
4.0
3.0
2.0
0
PPM (δ) 60MHz

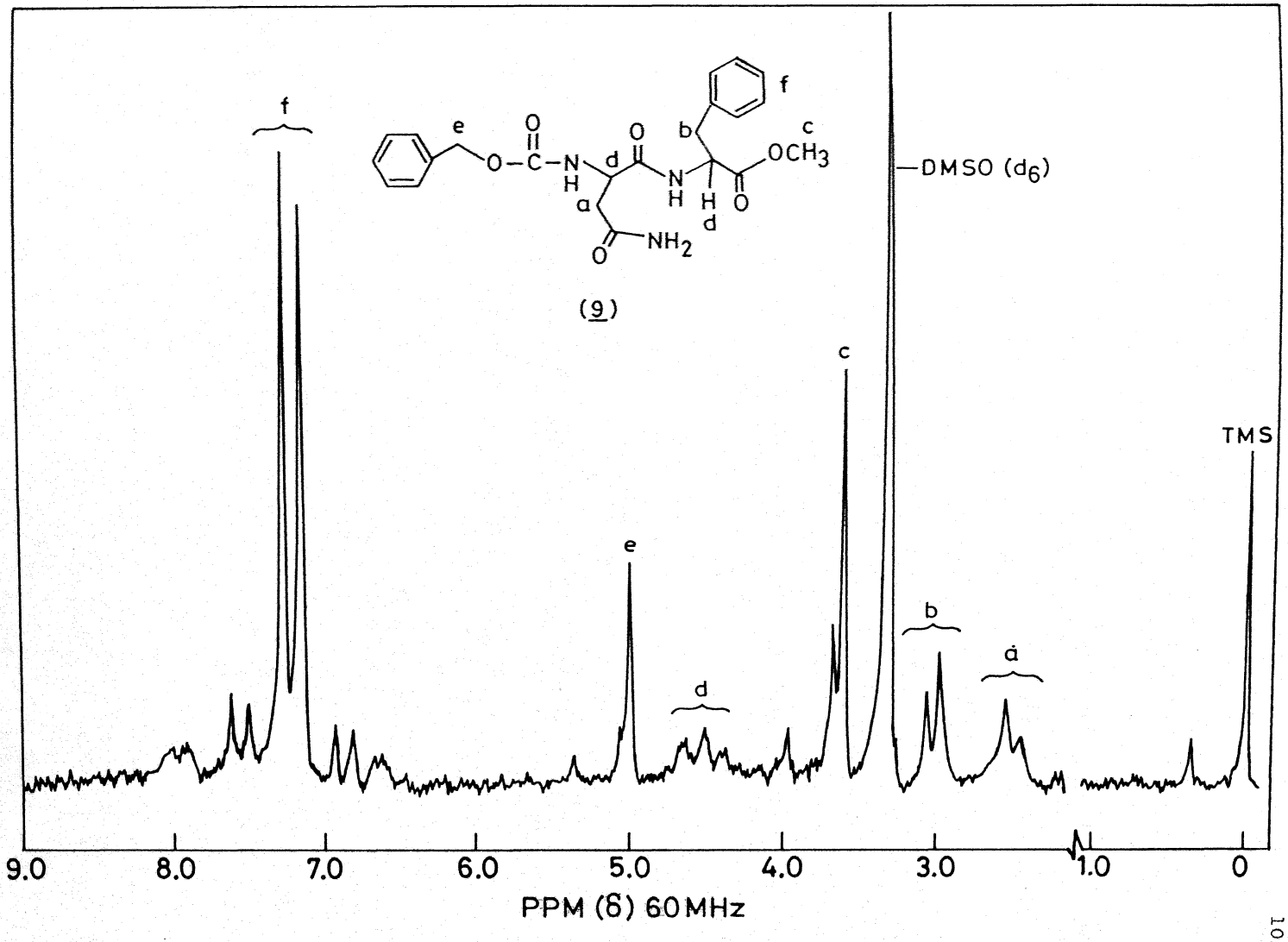

f
e
b
H
H
O
O-C-N
N
OCH3
O
a
H
H
O
d
c
c
H2N
(4b)
d
a+b+c
TMS
f
e
9.0
8.0
7.0
6.0
5.0
4.0
3.0
1.0
0
PPM (δ) 60 MHz

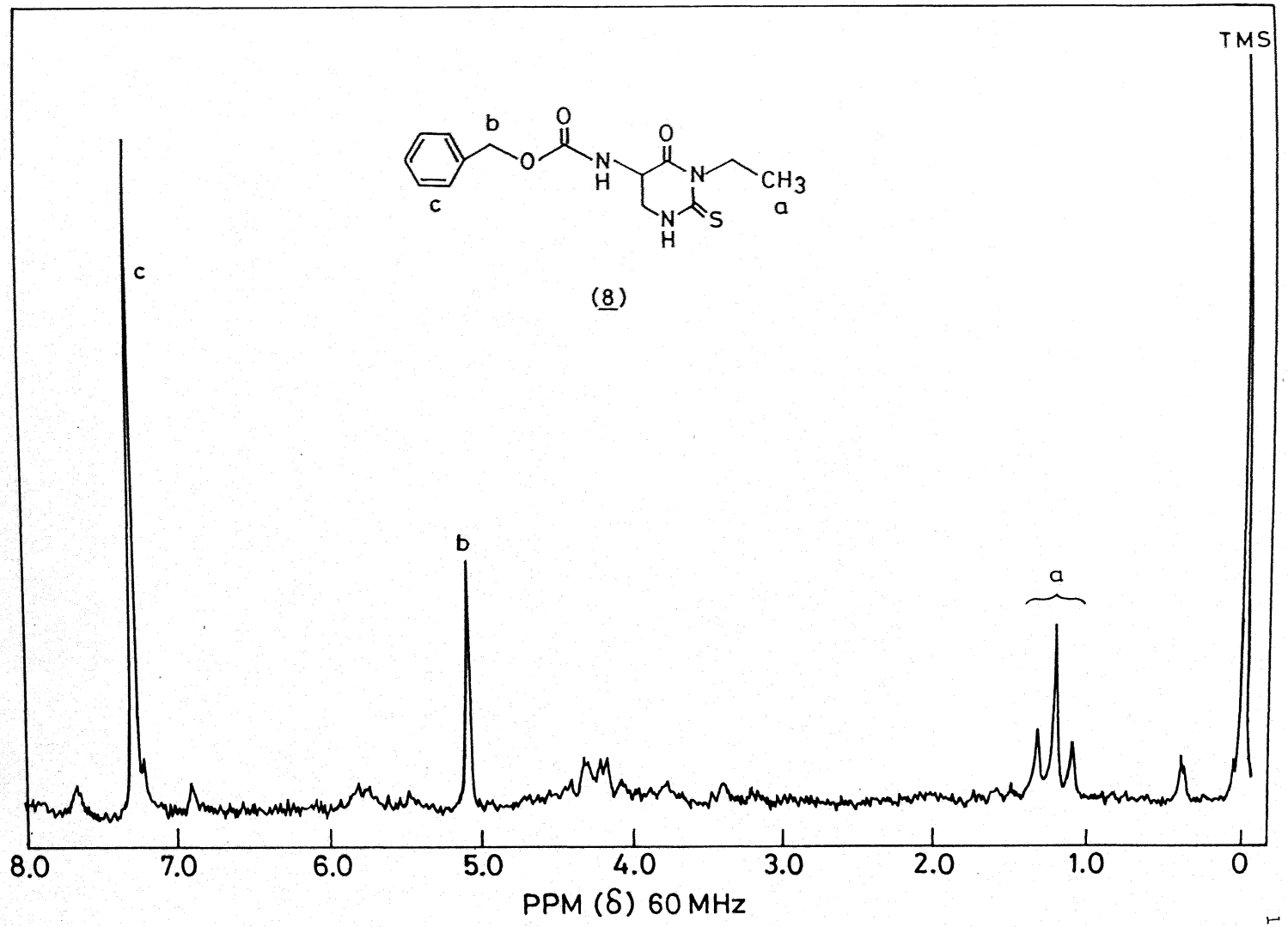

e
d
b
CH2–O–C–NH
COOCH3
H
CH2
c
a
O
NH2
e
(5)
TMS
b
d
a
c
9.0
8.0
7.0
6.0
5.0
4.0
3.0
1.0
0
PPM (δ) 60MHz

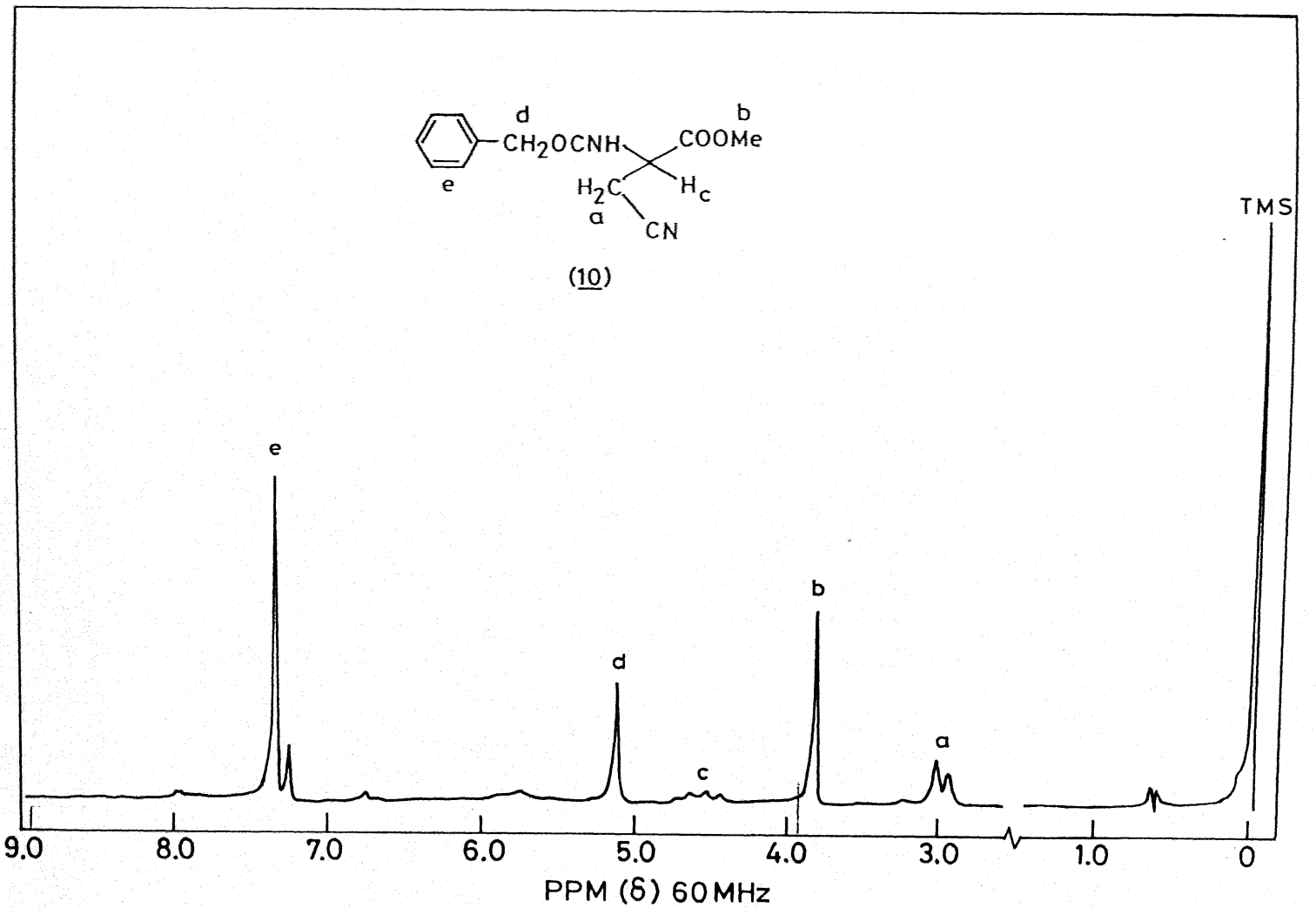

TMS
f
e
d
c
b
a
(7c)
80
70
60
50
40
30
20
10
0
PPM (δ) 60 MHz

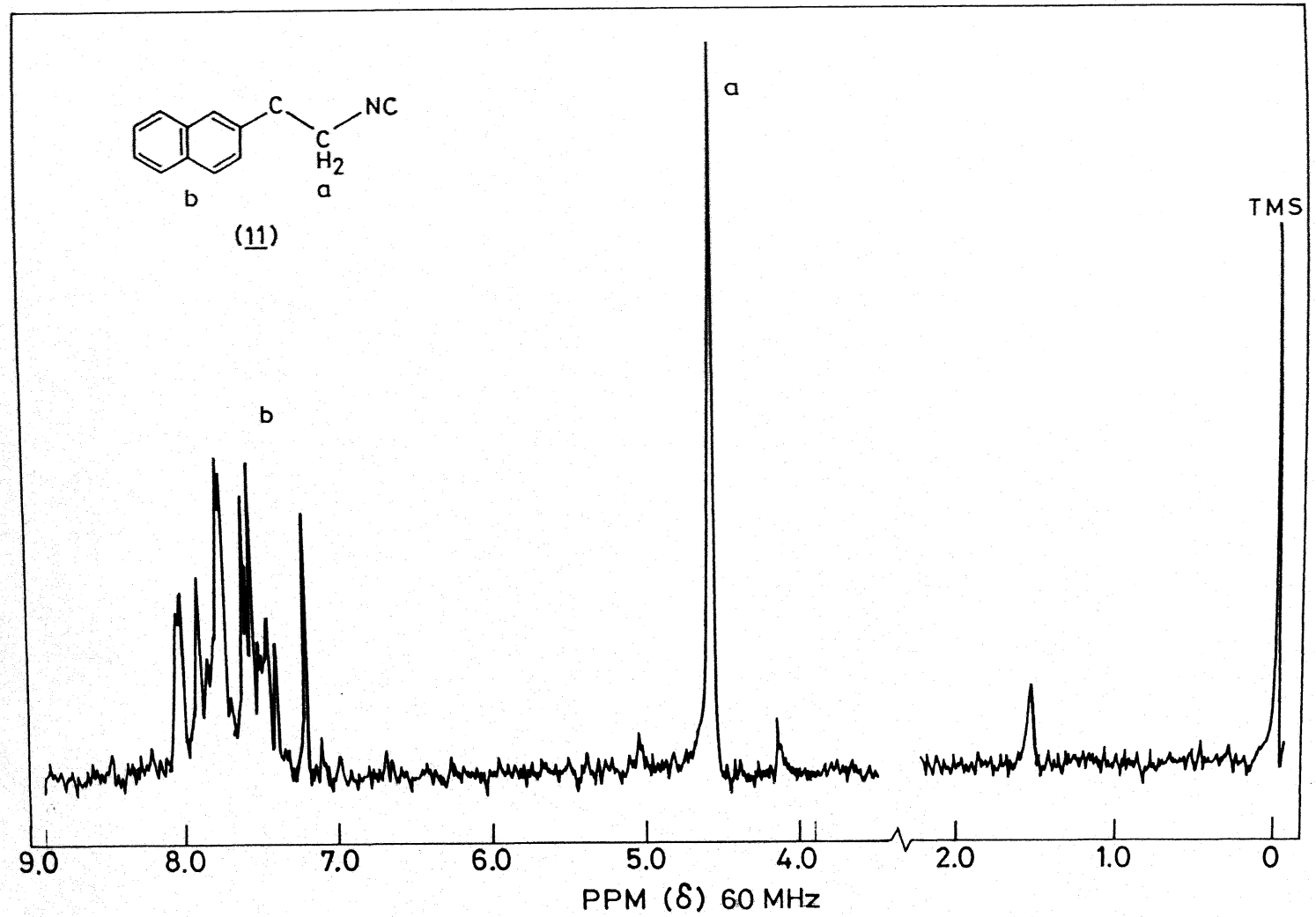

e
H
f
c
CH2OCONH
COOCH3
g
g
H2C
S
b a
d
N
NHCH2CH3
H
TMS
c
f
a
d
e
b
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ) 60 MHz

(7a)


f
e
O
O-C-N
H
d
O
N
H
b
f
c
OCH3
a
H
d
O
O
NH2
(9)
DMSO (d6)
c
TMS
e
d
b
a
9.0
8.0
7.0
6.0
5.0
4.0
3.0
1.0
0
PPM (δ) 60MHz


TMS
b
O
O
N
H
O
N
CH3
c
a
N
H
S
(8)
c
b
a
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ) 60 MHz


d
b
CH2OCNH
COOMe
e
H2C
Hc
a
CN
(10)
TMS
e
d
b
c
a
9.0
8.0
7.0
6.0
5.0
4.0
3.0
1.0
0
PPM (δ) 60 MHz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NC
C
H2
b
a
(11)
a
TMS
b
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7.0
6.0
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2.0
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0
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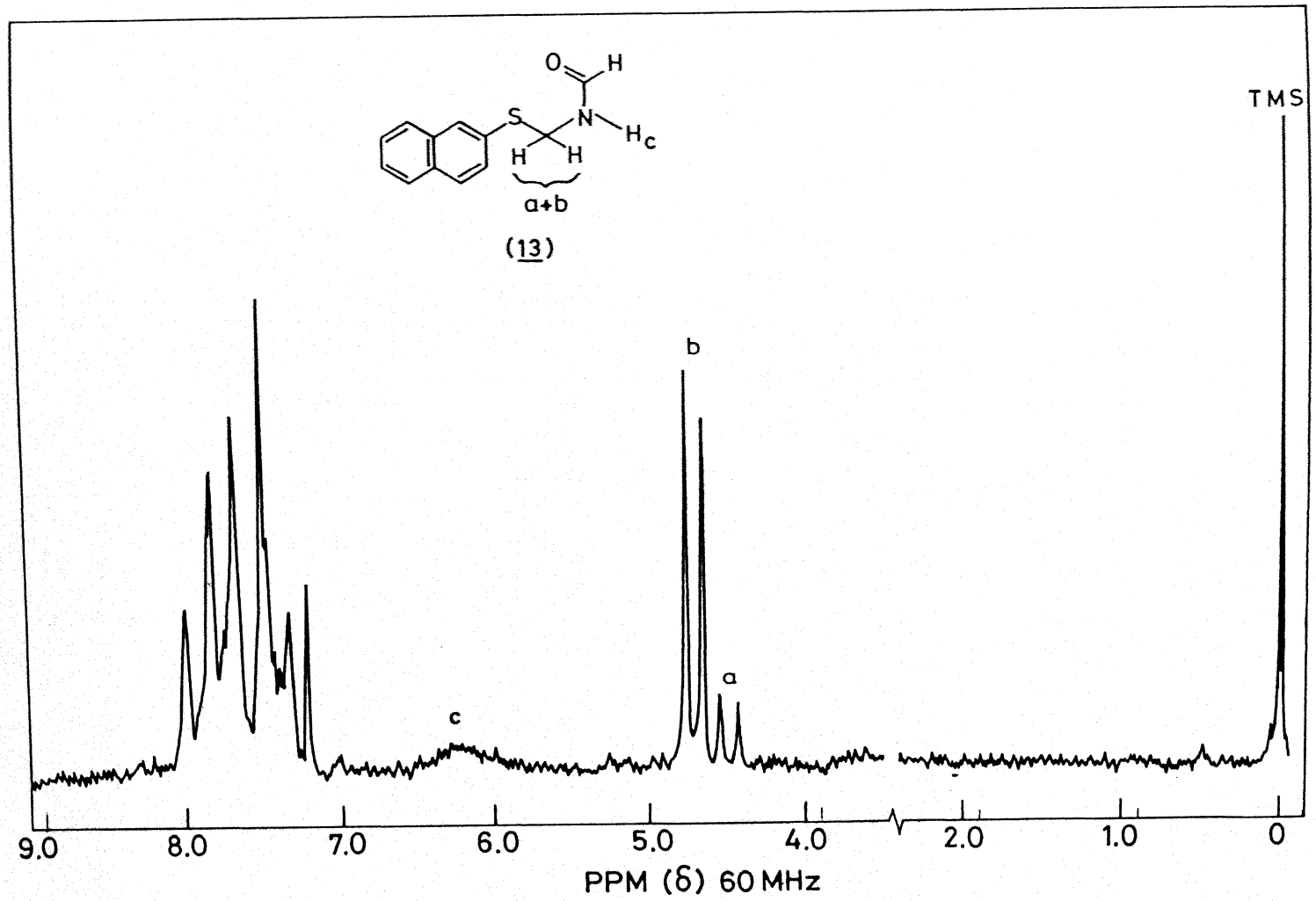

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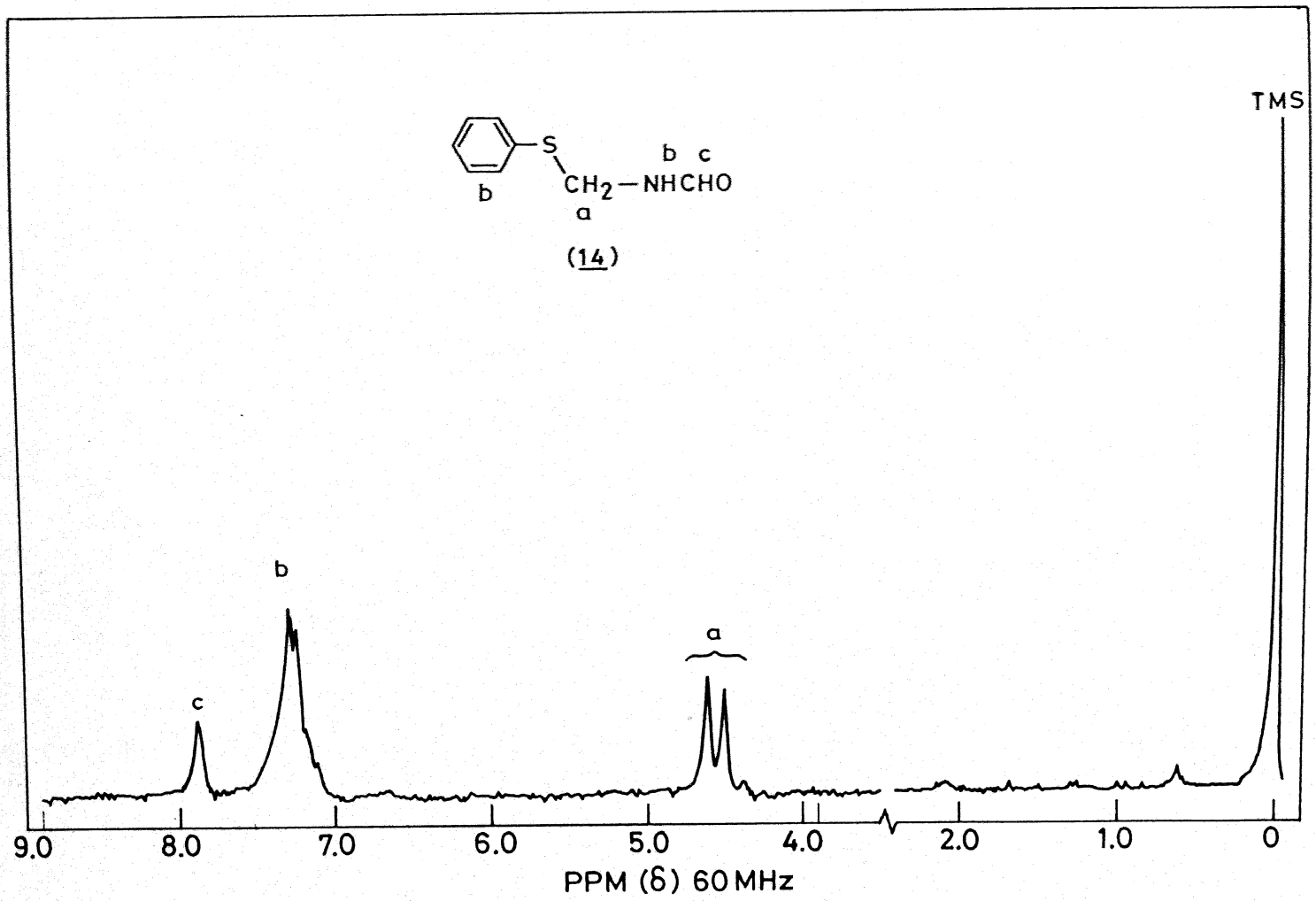
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b c
S
b
CH2 — NHCHO
a
(14)
b
c
a
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8.0
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5.0
4.0
2.0
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0
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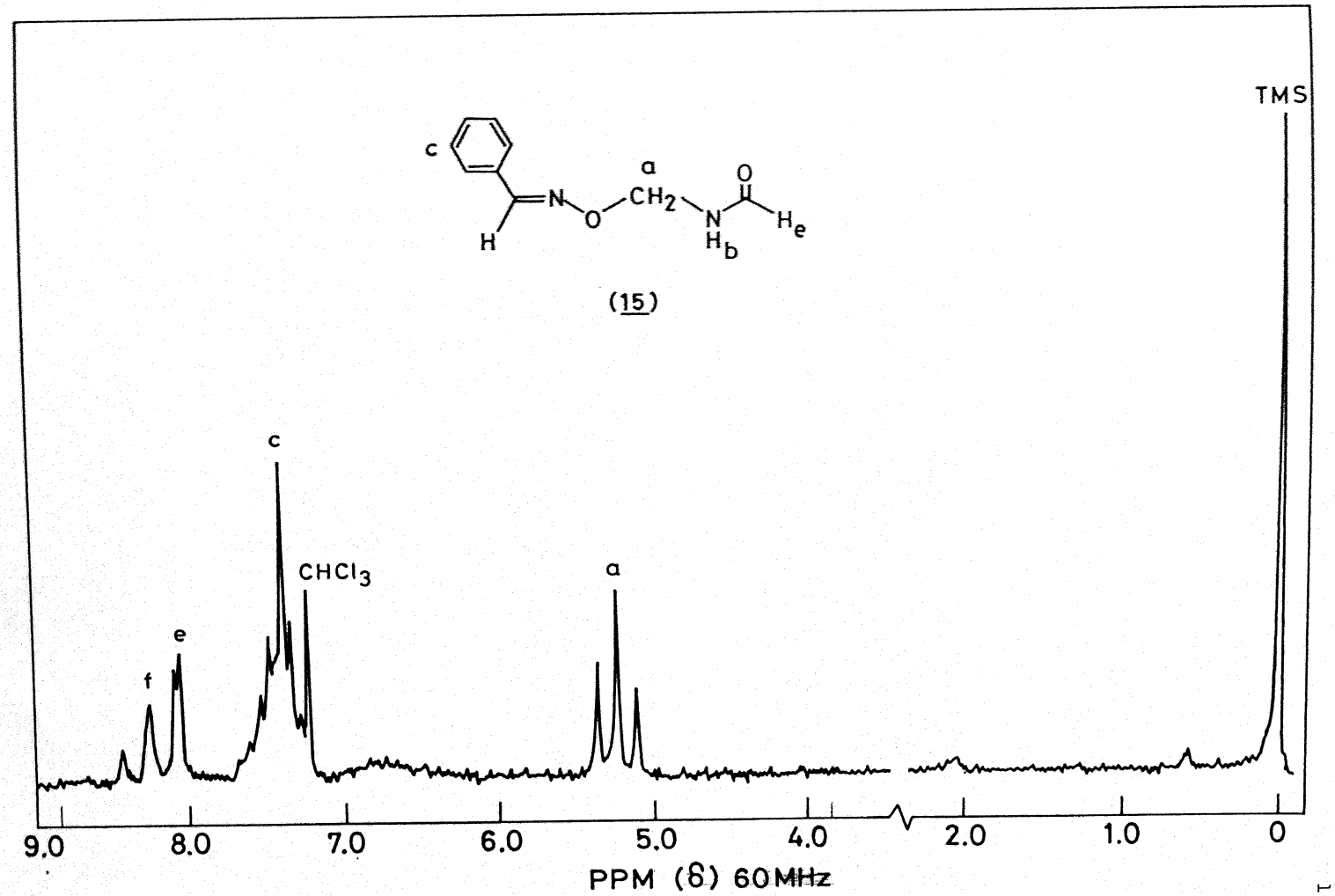
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O
N
O
CH2
N
H
Hb
He
(15)
c
CHCl3
a
e
f
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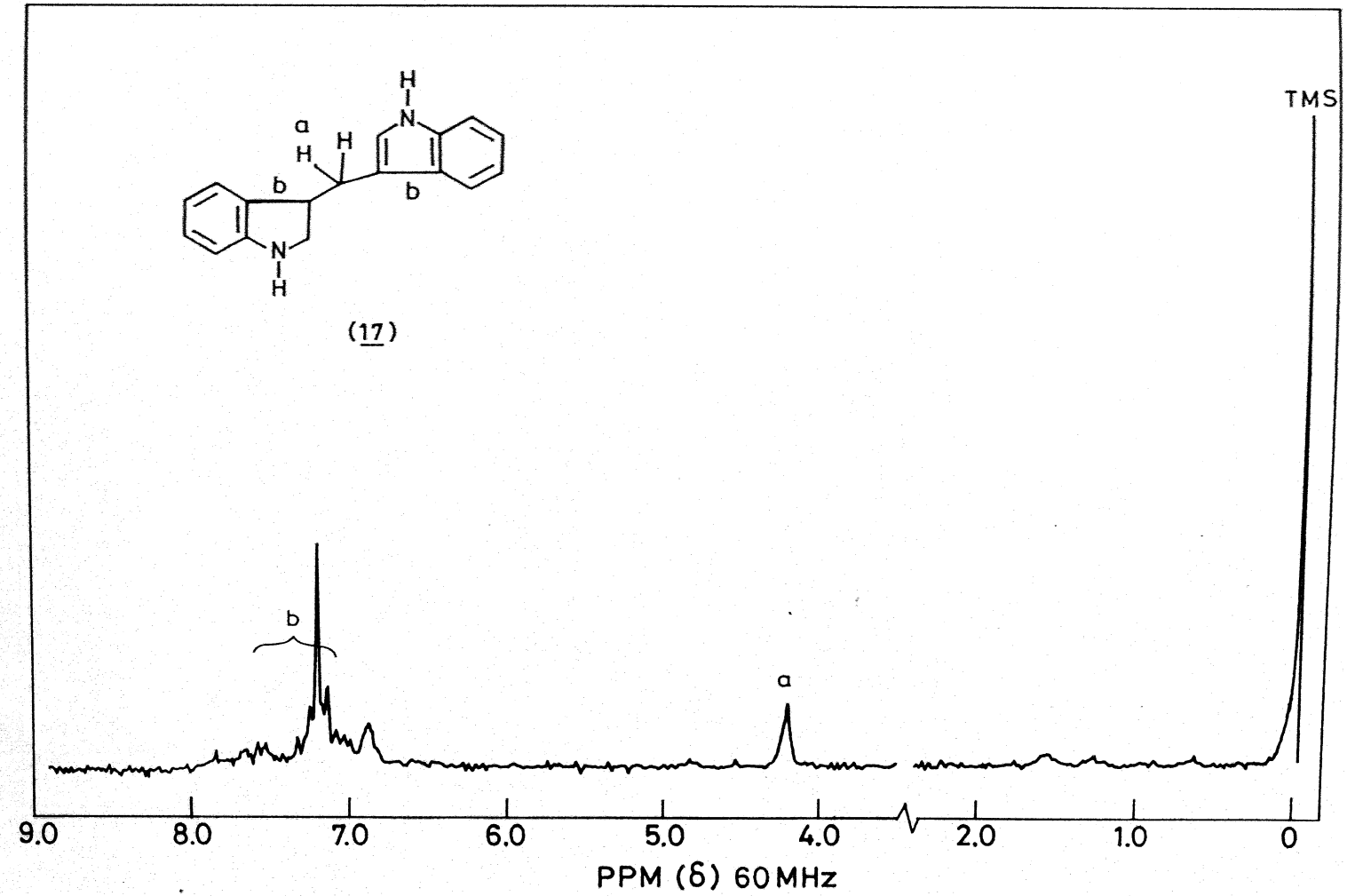
8.0
7.0
6.0
5.0
4.0
2.0
1.0
0
PPM (δ) 60MHz


H
N
a
H
H
b
b
N
H
(17)
TMS
b
a
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8.0
7.0
6.0
5.0
4.0
2.0
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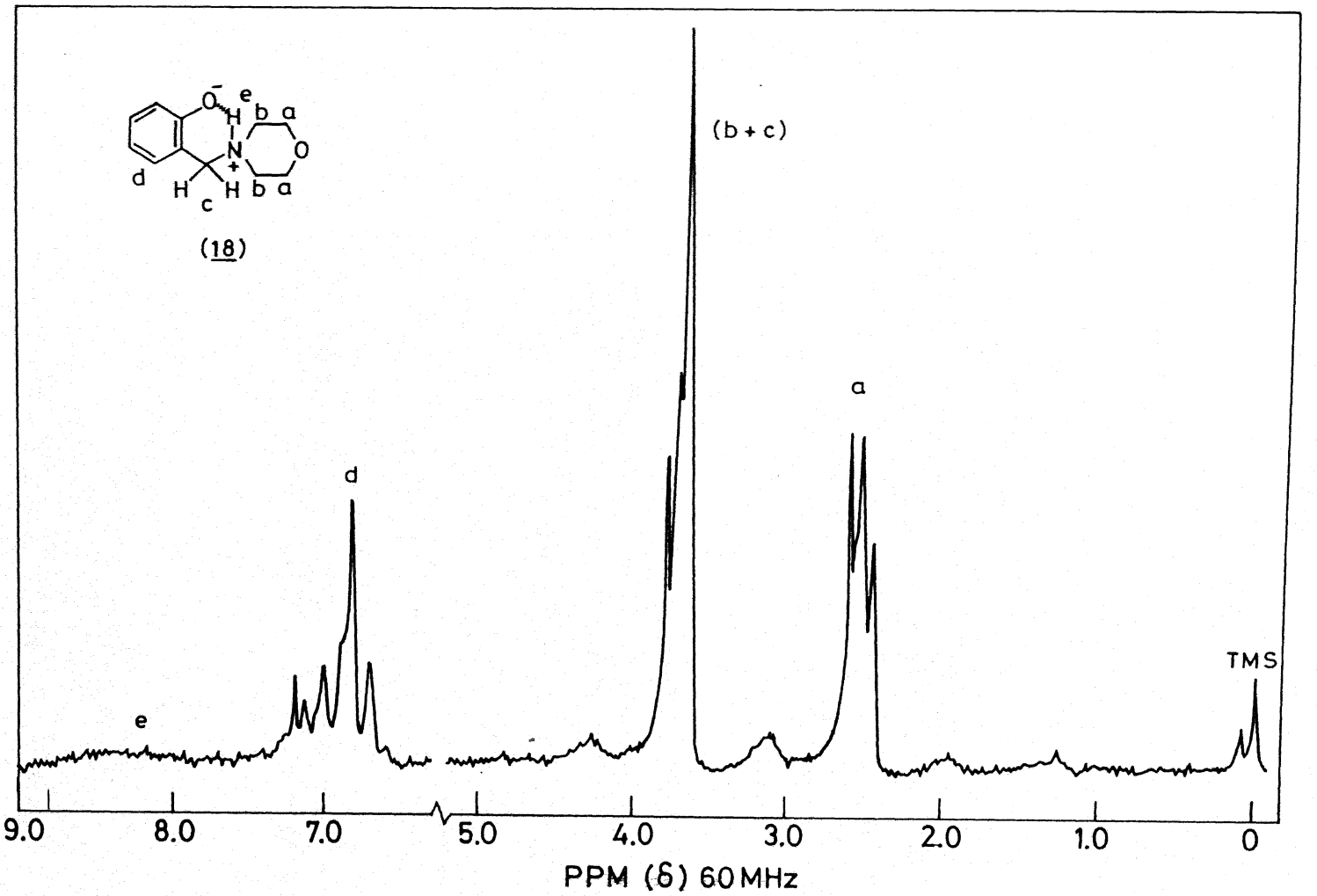
0
PPM (δ) 60 MHz



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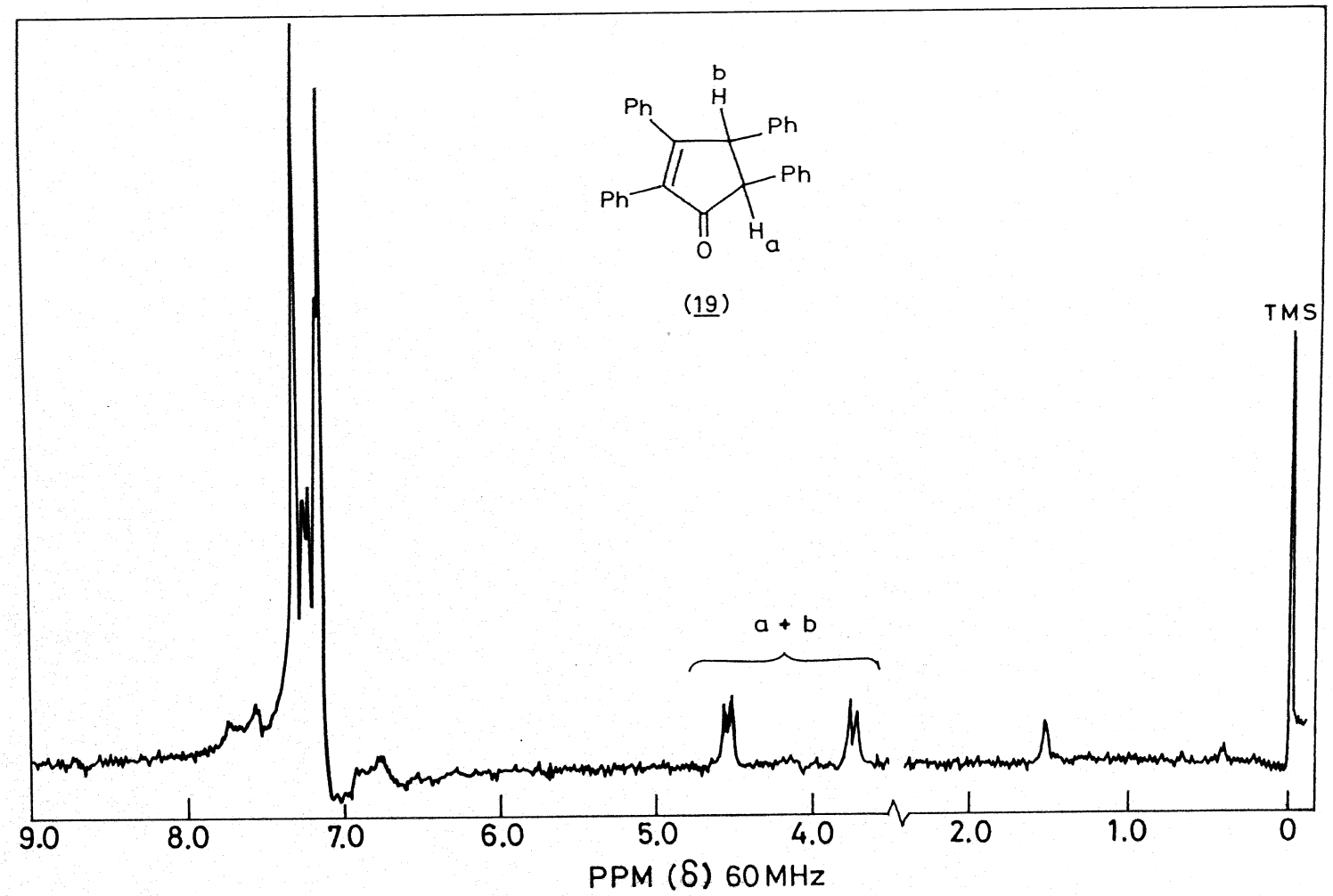
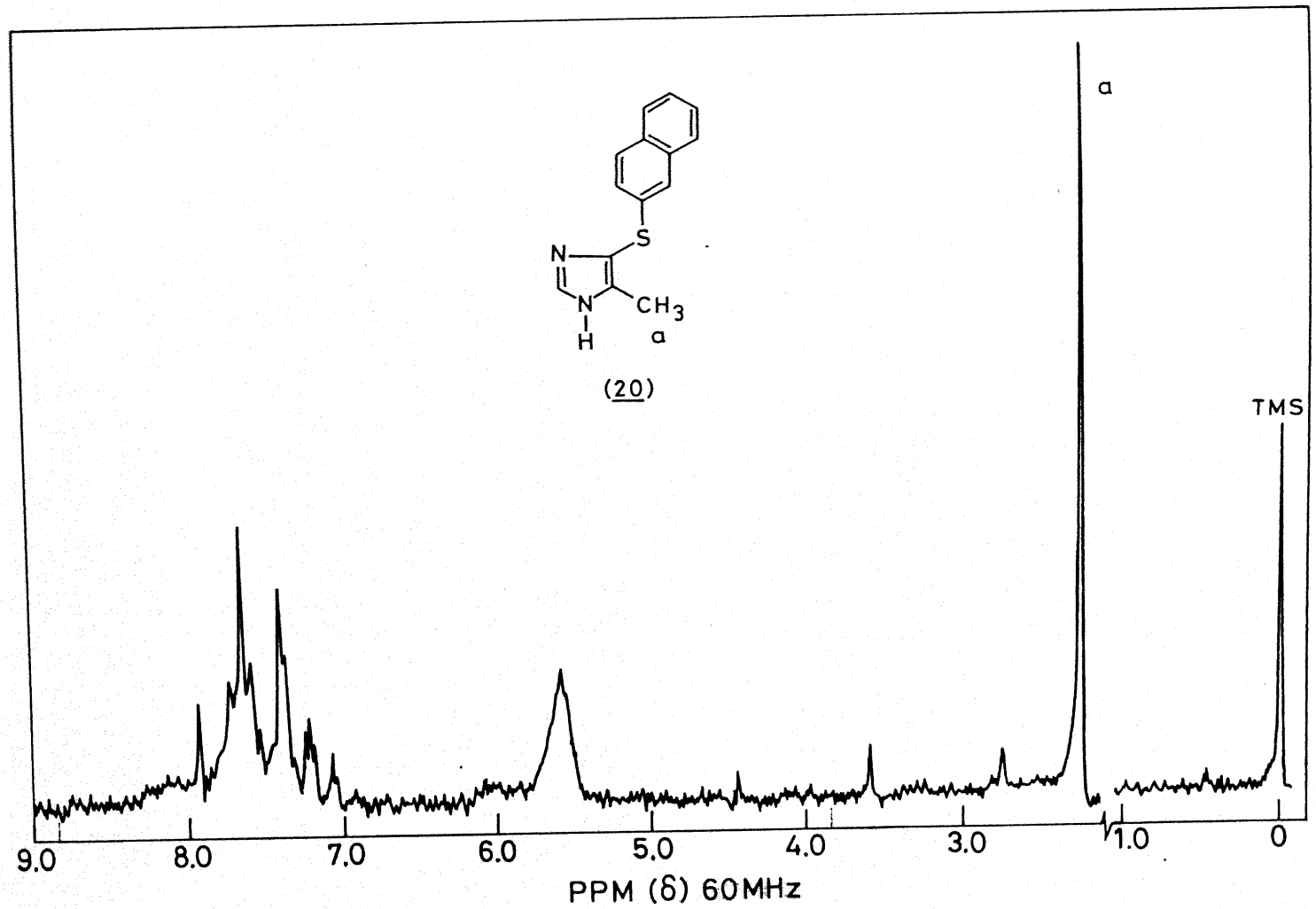
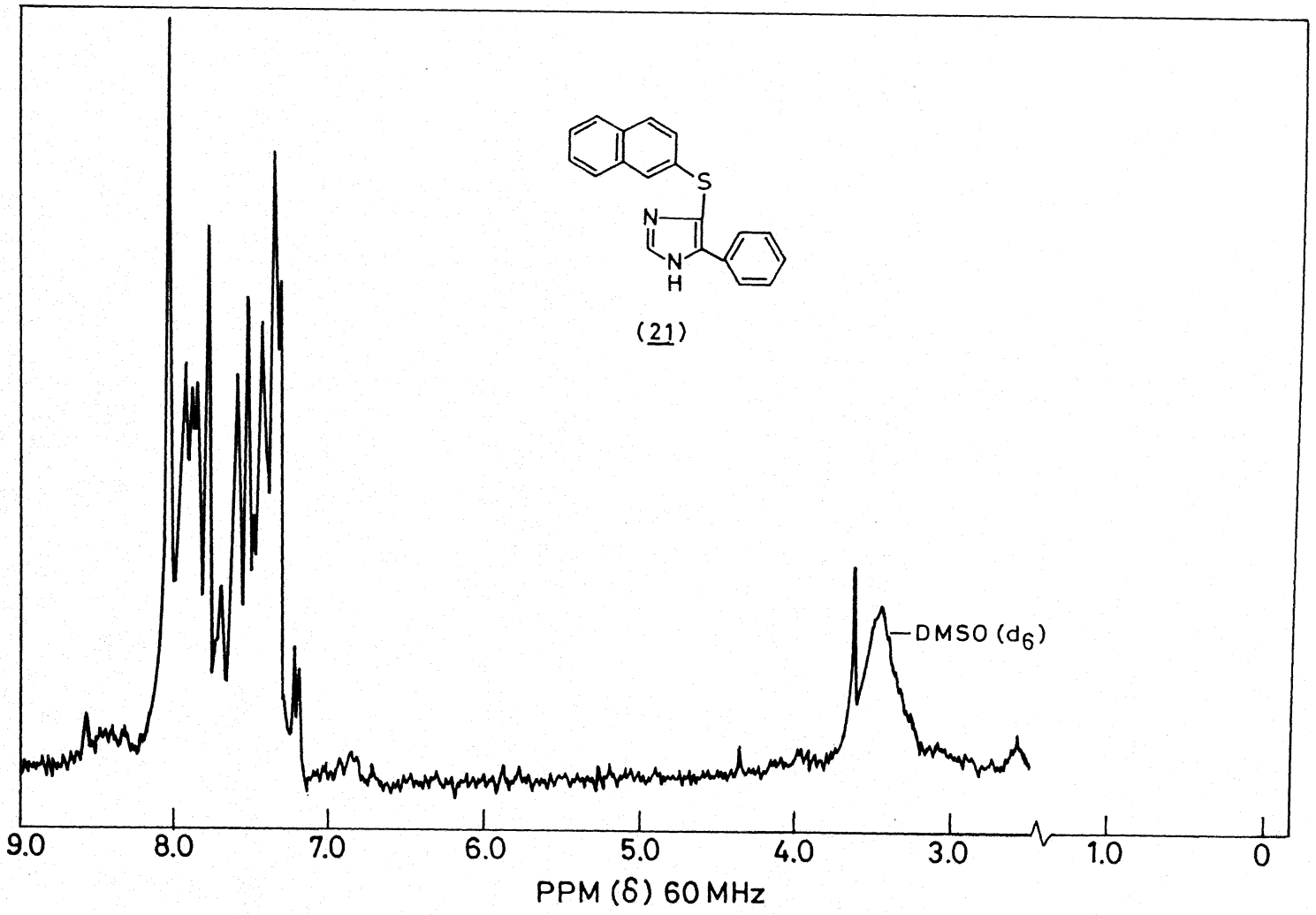

b
H
Ph
Ph
Ph
Ph
H a
O
(19)
TMS
a + b
9.0
8.0
7.0
6.0
5.0
4.0
2.0
1.0
0
PPM (δ) 60 MHz



(20)



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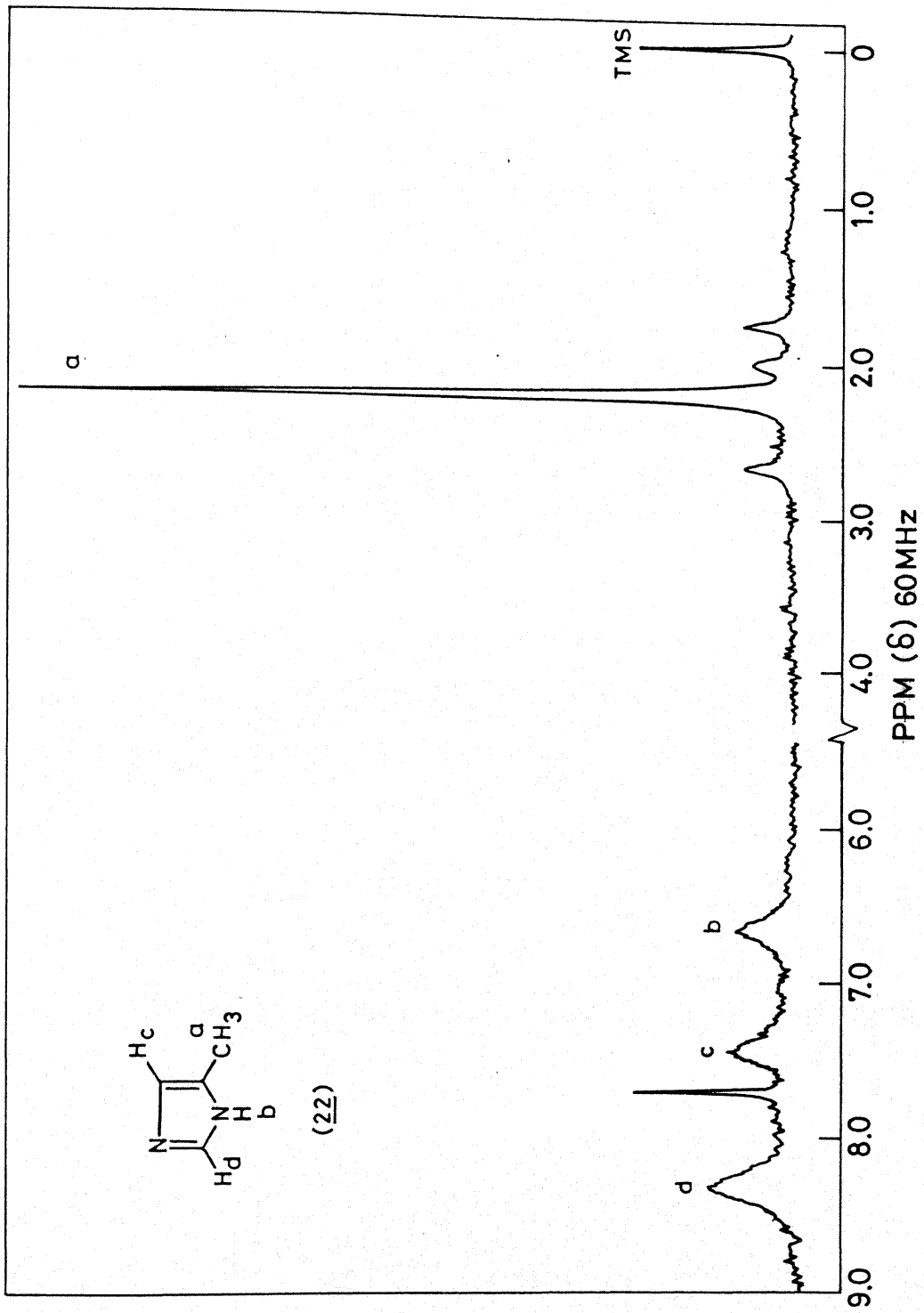
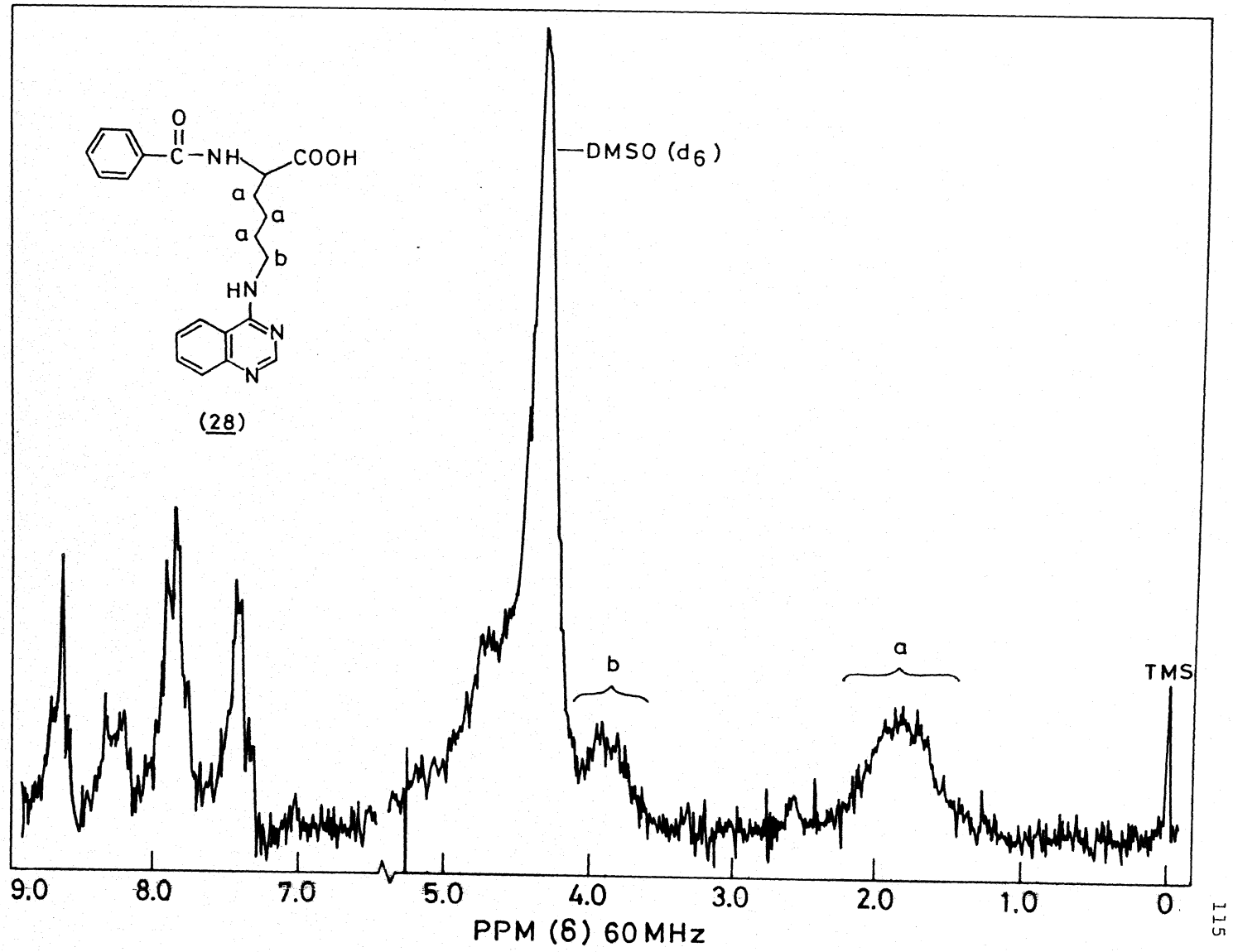


(22)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C–NH
COOH
a
a
a
b
HN
N
N
(28)
DMSO (d6)
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TMS
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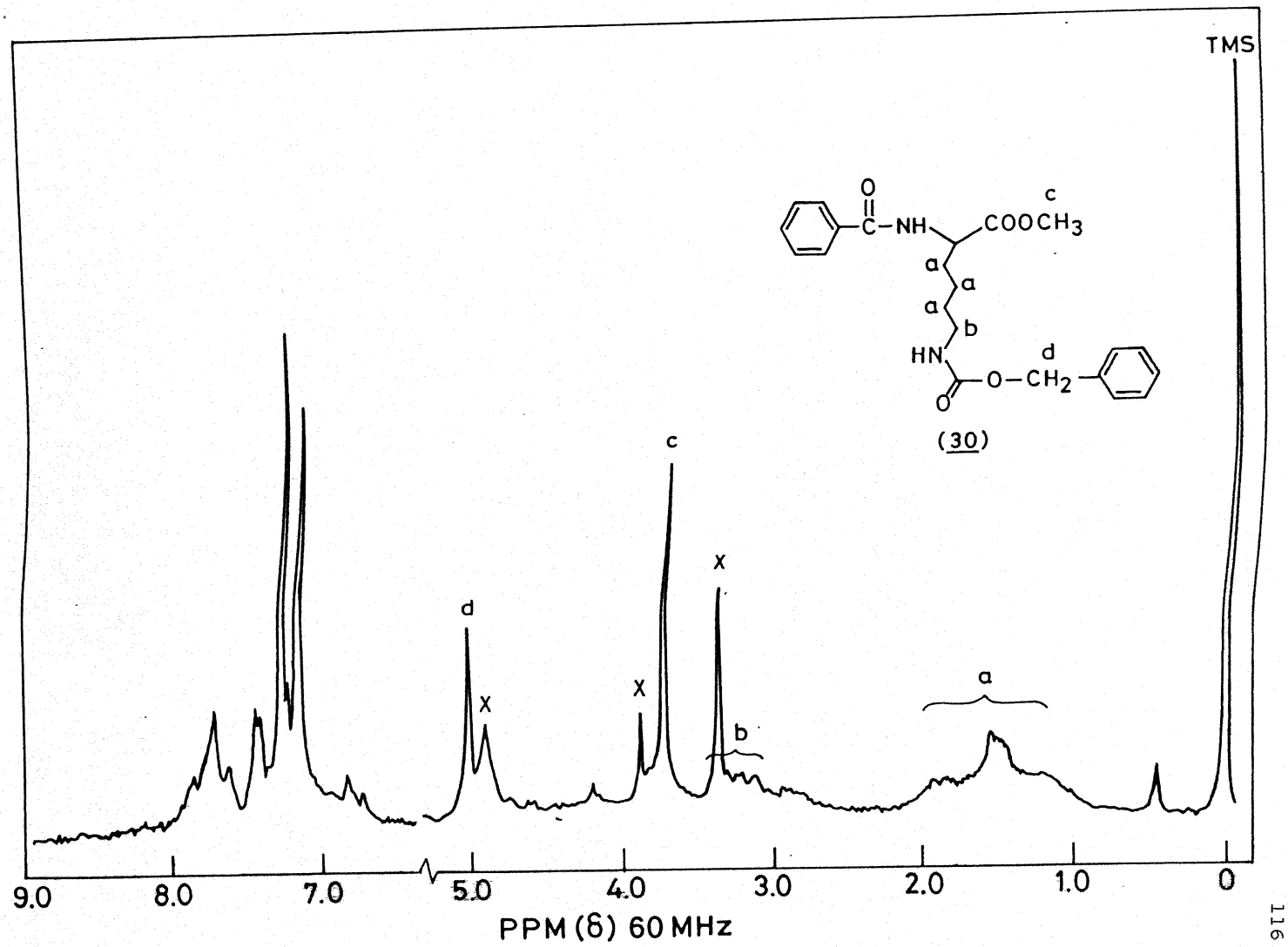
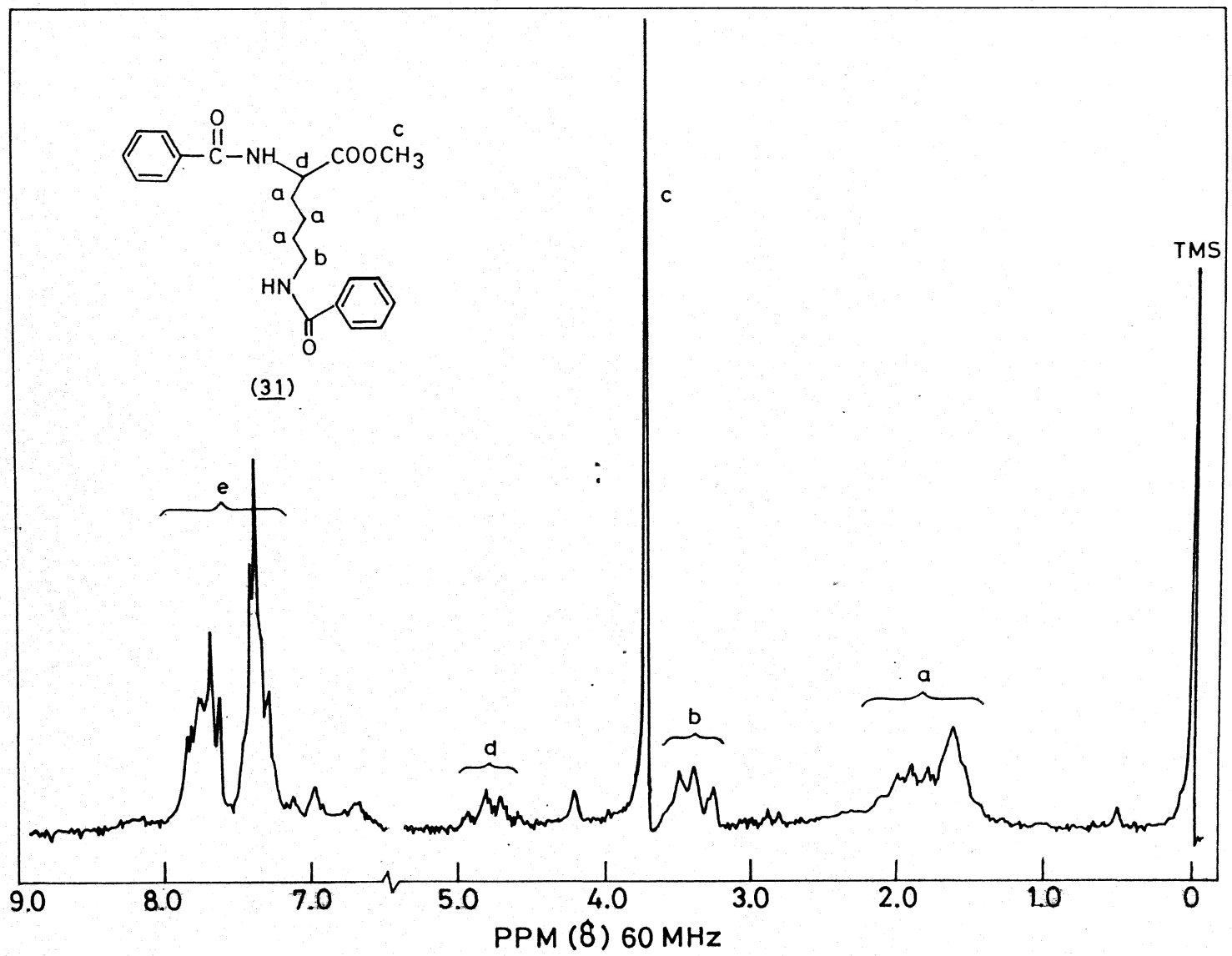
8.0
7.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ) 60 MHz


TMS
O
C–NH
c
COOCH3
a
a
a
b
HN
d
O–CH2
O
(30)
c
X
d
X
X
b
a
9.0
8.0
7.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ) 60 MHz


O
C–NH
d
COOCH3
c
a
a
a
b
HN
O
(31)
e
d
c
b
a
TMS
9.0
8.0
7.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ) 60 MHz

SECTION E. EXPERIMENTAL

Melting points and boiling points are uncorrected. Infrared spectra were recorded on Perkin-Elmer Model 580 spectrophotometer either as neat liquids or as thin KBr wafers. NMR spectra were obtained on dilute solution in $CDCl_3$ or CCl_4 or DMSO-D_6 on Hitachi R600 (FT) spectrometer. The chemical shifts are reported in ppm downfield from internal TMS at 0.00 as standard. Mass spectra were recorded with a Jeol instrument. Elemental analysis were carried out in automatic C, H, N analysers. Silica gel G (Merck) was used for tlc and column chromatography was done on silica gel (acme, 100-200 mesh) columns, which were invariably made from a slurry in benzene. Reactions were monitored, wherever possible, by tlc.

1. Reaction of benzyloxycarbonyl Chloride and L-glutamine : Preparation of benzyloxycarbonyl glutamine (Z-Gln).

Benzyloxycarbonyl chloride (95%), (7.3 ml, 48 mmol) in dioxane (14 ml) and 4N NaOH (8.3 ml) was added in five equal lots over 1.5h, to a stirred and ice cooled solution of L-glutamine (4.87g,33.33 mmol) in 4N NaOH (10 ml) and 1N $NaHCO_3$(33 ml). The medium was kept basic throughout. The mixture was left stirred for 0.75h, extracted with EtOAc (1x25 ml), the aqueous layer acidified using 4N HCl, extracted with EtOAc (2x50 ml), dried and evaporated to yield 8.5g (91%) of Z-Gln. mp 135°C,(lit mp. 133-137°C).

IR : ν_{max}(KBr) cm^{-1}: 3500,3370(-NH),1750, 1700, 1610, 1530 (acid, amide).

II. Esterification of Z-Gln : Preparation of Z-glutamine methyl ester (Z-Gln-OMe, 1a).

Z-Gln (3.5g,12.5 mmol),in MeOH(10 ml) was admixed with ethereal diazomethane, prepared from 6g of N-nitrosomethyl urea and 20% aq. KOH(40 ml). The mixture was refrigerated over-night, evaporated and crystallized from EtOAc/Hexane to give Z-Gln-OMe (3.7g,87%).

(1a) : mp 138°C. (lit mp. 140-141°C).

IR : ν_{max}(KBr) cm^{-1}: 3440, 3340 (-NH),1750 (ester), 1650, 1550 (amide).

NMR : $\delta(CDCl_3 + DMSO\text{-}d_6)$: 2.15 (m, 4H, $-C\underline{H}_2-C\underline{H}_2-\overset{O}{\overset{\|}{C}}-$),

3.6 (s, 3H, $-OC\underline{H}_3$),4.5(m, 1H, tertiary proton),

5.0 (s, 2H,$-O-C\underline{H}_2-Ph$),7.3(s, 5H,aromatic).

III. Reaction of Z-Gln with p-nitrophenol : Preparation of Z-glutamine p-nitrophenyl ester (Z-Gln-ONP,1b).

Under ice cooling and stirring,a solution of Z-Gln (4.26g,15 mmol) and p-nitrophenol (2.52g, 18 mmol) in dry DMF (25 ml) was admixed with DCC (3.06g,15 mmol), left stirred 3h, refrigerated overnight, filtered, washed with DMF (3 ml), the combined filtrates diluted with H_2O(150 ml), the resulting solid washed with cold water and dried to yield 5.7g (93%) of crude Z-Gln-ONP, mp. 150°C (lit mp. 155°C) which was used as such for all reactions.

IV. Reaction of Z-Gln with p-nitrobenzyl alcohol : Preparation of Z-glutamine p-nitrobenzyl ester (Z-Gln-O-Bzl-p-NO_2,1C).

A stirred mxiture of Z-Gln (1.4g, 5 mmol), p-nitrobenzyl bromide (1.972g, 7.5 mmol), and Et_3N (1.04 ml, 7.5 mmol) in EtOAc (20 ml) was refluxed for 12 h,filtered hot, admixed with 5% aq. MeOH (50 ml), the precipitated solid collected, washed with cold H_2O(20 ml), 1N HCl (2x20 ml), H_2O(20 ml), 1M $KHCO_3$(3x20ml), saturated NaCl (3x20 ml) dried and crystalized from EtOAc/Hexane to give 1.24g,(62%) of Z-Gln-O-Bzl-p-NO_2,mp. 133°C (lit. mp.135°C).

IR : ν_{max}(KBr) cm^{-1}: 3500, 3350, (-NH), 1750 (ester), 1665, 1620,1540 (amide).

NMR : $\delta(CDCl_3 + DMSO\text{-}d_6)$,2.20 (m, 4H, $-C\underline{H}_2-C\underline{H}_2-\overset{O}{\overset{\|}{C}}$), 4.2(m,1H,tertiary proton),5.10 (s,2H,$-O-C\underline{H}_2-Ph$), 5.25 (s,2H, $-O-C\underline{H}_2-Ph(p-NO_2)$),7.3-8.2 (m, 9H, aromatic).

V. Preparation of benzyloxycarbonyl glutaminyl phenyl alanine methyl ester (Z-Gln-Phe-OMe,2b).

a. Phe-OMe.HCl :

Thionyl chloride (4.9 ml, 67 mmol) was added in drops to stirred and ice cooled dry methanol (44 ml) followed by L-phenyl alanine (9g, 54.5 mmol). The reaction mixture was refluxed for 2 h, solvents evaporated, and the residue on crystalization from dry MeOH/dry-ether gave 8.5g, (72%) of Phe-OMe.HCl, mp. 160°C (lit. mp. 160°C).

b. Z-Gln-Phe-OMe : (2b)

To a stirred solution of Phe-OMe.HCl (1.401g, 6.5 mmol) in dry pyridine (30 ml) was added (1b) (8.406g, 6.5mmol) followed by Et_3N(0.9 ml, 6.5 mmol). The reaction mixture was left stirred overnight, concentrated in vacuo, diluted with water (50 ml), the precipitated solid filtered, washed with large amounts of

H_2O, 10% $NaHCO_3$ (50 ml), H_2O and dried, to give 2.6g (96%) of Z-Gln-Phe-OMe, (2b).mp. 180°C.

IR : ν_{max}(KBr) cm^{-1}: 3340, 3320 (-NH), 1750 (ester), 1700 (sh), 1665,1615 (sh), 1550 (amide).

NMR : $\delta(CDCl_3 + DMSO\text{-}d_6)$: 2.15(m, 4H, $-CH_2-CH_2-\overset{O}{\overset{\|}{C}}$),3.0(d, 2H, J=7Hz,$-CH_2-Ph$), 3.65(s, 3H, $-O-CH_3$), 4.2(m,1H, tertiary proton), 4.6(m,1H, tertiary proton), 5.0 (s,2H,$-O-CH_2-Ph$),7.15 (s,5H, aromatic), 7.25(s, 5H, aromatic).

ms : m/z: 441(M^+).

VI. Preparation of benzyloxycarbonyl glutaminyl glycine methyl ester (Z-Gln-Gly-OMe, 2a).

a. Gly-OMe.HCl :

Under stirring, dry HCl was passed through an ice cooled suspension of L-glycine (10g, 137 mmol) in MeOH (150 ml) till saturation. The reaction mixture was allowed to attain rt, subjected to dry HCl treatment for another 0.5h, when a clear solution was obtained, solvents were evaporated and the residue on crystalization from MeOH/dry ether gave Gly-OMe.HCl(15g, 89%). mp. 175°C.

b. Z-Gln-Gly-OMe: (2a)

Under conditions described in EXPERIMENT Vb, Gly-OMe.HCl (3.4 mmol) and (1b) (3.15 mmol) gave Z-Gln-Gly-OMe (2a), (0.5g,44%), mp 172°C.(lit mp 174°C).

IR : ν_{max}(KBr) cm^{-1}: 3460, 3340 (-NH), 1750 (ester), 1665,1615,1555 (amide).

NMR : $\delta(CDCl_3 + DMSO\text{-}d_6)$ 2.15 (m,4H, $-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-$), 3.65 (s, 3H, $O-CH_3$), 3.9 (d, 2H, J=7Hz, $-NH-CH_2-CO-$),4.2 (m,1H, tertiary proton), 5.05(s, 2H, $-O-CH_2-Ph$), 7.3(s,5H, aromatic).

VII. Preparation of benzyloxycarbonyl glutaminyl leucine methyl ester (Z-Gln-Leu-OMe,2C).

a. Leu-OMe.HCl :

Under stirring dry HCl was passed through a suspension of L-leucine (5g,38 mmol) in dry methanol (30 ml) for 2 h, the resulting clear solution evaporated in vacuo and the residue on crystalization from dry MeOH/dry ether gave Leu-OMe.HCl(6g, 88%), mp. 149°C (lit. mp. 151°C).

b. Z-Gln-Leu-OMe : (2C) :

From (1b) (3 mmol) and Leu-OMe.HCl(3.3 mmol), by procedure described in EXPERIMENT Vb, was obtained Z-Gln-Leu-OMe, (2C) (1g,83%),mp.162°C (lit.mp. 164°C).

IR : ν_{max}(KBr) cm^{-1}: 3460,3340 (-NH),1740 (ester),1690, 1665, 1620 (sh), 1550 (amide).

NMR : δ($CDCl_3$+ DMSO-d_6) 0.95 (d, 6H, $-CH(CH_3)_2$), 1.6 (m, 3H, $-CH_2-CH$), 2.15(m, 4H, $-CH_2-CH_2-C(=O)-$), 3.65(s, 3H, $-OCH_3$), 4.2(m, 2H, tertiary protons), 5.05(s, 2H, $-O-CH_2-Ph$), 7.3 (s, 5H, aromatic).

ms : m/z : 407 (M^+).

VIII. The reaction of Iodobenzene with acetic anhydride and H_2O_2: Preparation of $PhI(OAc)_2$:

To stirred H_2O_2(30%, 30 ml) was added, in drops, acetic anhydride (102 ml). The reaction mixture was stirred overnight at 40°C, admixed dropwise with iodobenzene (16.5g, 81 mmol), left stirred overnight, poured onto hexane (500 ml), stirred and decanted. Addition of ether (50 ml) gave white solid

that was filtered and dried to give $PhI(OAc)_2$ (18g, 70%), mp.162° (lit.mp. 164°).

IX. Preparation of $PhI(TFA)_2$:

A solution of $PhI(OAc)_2$(5g), in warm TFA(10 ml) was left aside for 2 h at rt, filtered and dried to give $PhI(TFA)_2$(4.5g, 68%), mp. 122°C.(lit.mp. 126°C). The compound was immediately used for the next reaction.

X. The reaction of N-benzyloxycarbonyl glutamine methyl ester (Z-Gln-OMe,1a) with $PhI(TFA)_2$: Isolation of cyclic lactam(3) :

A stirred suspension of $PhI(TFA)_2$ (1.078g, 2.5 mmol) in $CH_3CN:H_2O$::1:1 (14 ml), pyridine (0.3 ml, 3.4 mmol) was admixed with (1a), (0.5g,1.7 mmol), left stirred 3 h, rt, concentrated in vacuo diluted with H_2O (20 ml), extracted with EtOAc (2x25 ml), dried, evaporated, dissolved in CH_2Cl_2(15 ml), left stirred with saturated solution of $NaHCO_3$(20 ml) for 3h, layers separated, dried, evaporated, and the residue on crystalization from EtOAc gave (0.22g,55%) of cyclic lactam (3) mp 172°C.(lit.mp.178°C).

IR : ν_{max}(KBr) cm^{-1}: 3270 (-NH), 1670,1650, 1540 (amide).

NMR : δ ($CDCl_3$+ DMSO-d_6) : 3.30 (m, 4H, $-C\underline{H}_2-C\underline{H}_2-NH$),

4.2(m, 1H, tertiary proton), 5.05 (s, 2H, $-OC\underline{H}_2-Ph$),

7.35 (s, 5H, aromatic).

ms : m/z : 234 (M^+).

XI. Reaction of N-benzyloxycarbonyl glutamine p-nitrophenyl ester (Z-Gln-ONP,1b), with $PhI(TFA)_2$: Isolation of (3) :

The reaction of (1b) (0.7g, 1.75 mmol) with $PhI(TFA)_2$ (1.13g, 2.62 mmol), as described in EXPERIMENT X,followed by preparative TLC of the crude product using EtOAc as the developer gave (0.3g, 73%) of (3) mp. 172°C.

XII. Reaction of N-benzyloxycarbonyl glutamine-p-nitrobenzyl ester (Z-Gln-o-bzl(p-nitro), 1c) with $PhI(TFA)_2$: Isolation of (3) :

The reaction of (1c) (0.563g, 1.37 mmol) with $PhI(TFA)_2$ (0.875g, 2.03 mmol) as described in EXPERIMENT X, followed by crystalization of the crude product from CH_2Cl_2 gave 0.12g of (3), mp. 175°C.

The filtrate on evaporation and preparative TLC using EtOAc as developer gave additional 0.016g of (3) (total yield = 45%) and (0.064g, 18%) of cyclic glutamine mp. 110°C.

IR : ν_{max}(KBr) cm^{-1} : 3300(-NH), 1700, 1650, 1535(amide).

NMR : δ($CDCl_3$+ DMSO(d_6)) 2.15 (m, 4H, $-C\underline{H}_2-C\underline{H}_2-\overset{O}{C}$),

4.35 (m, 1H, tertiary proton), 5.0 (s, 2H, -O-C$\underline{H}_2$-Ph), 7.3 (m, 5H, aromatic).

ms : m/z : 262 (M^+).

XIII. Reaction of N-benzyloxycarbonyl glutaminyl glycine methyl ester (Z-Gln-Gly-OMe, 2a) with $PhI(TFA)_2$: Isolation of (4a).

A stirred suspension of $PhI(TFA)_2$(0.322g, 0.75 mmol) in DMF:H_2O::1:1 (6 ml) was admixed with (2a) (0.175g, 0.5 mmol) left stirred for 8 h, rt, concentrated in vacuo, admixed with cold water (10 ml), made acidic with cold HCl (2 ml), extracted with ether (20 ml), the aq. layer saturated with $NaHCO_3$, admixed with CH_2Cl_2(20 ml), left stirred overnight, the organic layer separated, dried evaporated to give (4a), (0.149g,90%) as an oil.

IR : ν_{max}(thin film) cm^{-1}: 3320 (-NH), 1750 (ester), 1690(br), 1550 (amide), 1540.

NMR : δ ($CDCl_3$),2.6-3.2 (m, 4H , -C$\underline{H}_2$-C$\underline{H}_2$-NH_2), 3.6 (s, 3H, -OC$\underline{H}_3$), 4.0 (m, 2H, tertiary protons, 5.10 (s, 2H, -OC$\underline{H}_2$-Ph), 7.25 (s, 5H, aromatic).

XIV. Reaction of N-benzyloxycarbonyl glutaminyl phenyl alanine methyl ester (Z-Gln-Phe-OMe,2b) with $PhI(TFA)_2$: Isolation of (4b).

A stirred suspension of $PhI(TFA)_2$(3.07g, 7.2 mmol) in DMF:H_2O::1:1 (36 ml) and pyridine (0.75 ml, 4.8 mmol) was admixed over 0.25 h with (2b), (2.1g, 4.8mmol), left stirred for 5 h, rt. diluted with H_2O (20 ml), extracted with ether, the aqueous layer evaporated in vacuo to give the crude amine (4b) (1.5g, 74%) as thick oil.

IR : ν_{max}(thin film) cm^{-1}: 3440, 3320 (-NH), 1750 (ester), 1690, 1610, 1515.

NMR : $\delta(CDCl_3)$: 2.9 (m, 6H, $-C\underline{H}_2-C\underline{H}_2-NH_2$ and $-C\underline{H}_2-Ph$), 3.61 (s, 3H, $-OC\underline{H}_3$), 4.4 (br, 2H, tertiary protons), 5.0 (s, 2H, $-O-C\underline{H}_2-Ph$), 7.05 (s, 5H, aromatic), 7.25 (s, 5H, aromatic).

The crude amine was dissolved in dry CH_2Cl_2, cooled and subjected to a dry stream of HCl, till saturation to give the hydrochloride, mp. 205°C.

XV. Preparation of benzyloxycarbonyl asparagine (Z-Asn) :

To a stirred and ice cooled 16% aq. salution of L-asparagine (2g, 15.15 mmol), benzyl chloroformate (50%), (6.3 ml, 18.2 mmol) and 2N NaOH (15 ml) was added over 1.5 h, in separate batches. The medium was kept basic throughout. The reaction mixture was left stirred for 1 h, extracted with ether (40 ml), acidified using 4N HCl, filtered, dried and crystalized from MeOH to yield 3.2g (80%) of Z-Asn, mp. 162°C.(lit.mp. 164-165°C).

IR : ν_{max}(KBr) cm^{-1}: 3440, 3370 (-NH), 1710, 1655, 1595, 1545 (amide).

XVI. Esterification of Z-Asn : Preparation of Z-asparagine methyl ester (Z-Asn-OMe, 5).

To an ice cooled solution of Z-Asn (3g, 11.70 mmol) in MeOH(20 ml) was added ethereal CH_2N_2-prepared from N-nitroso N-methyl urea (5g) and 40% KOH(20 ml), the reaction mixture refrigerated overnight, solvents evaporated and the residue crystalized from MeOH/Ether to give 2.0g (64%) of Z-Asn-OMe, mp. 150°C. (lit.mp. 156°C).

IR : ν_{max}(KBr) cm^{-1}: 3440, 3320 (-NH), 1750 (ester), 1685, 1560 (amide).

NMR : $\delta(CDCl_3 + DMSO - d_6)$: 2.7 (d, 2H, J=7 Hz, $-\underline{CH_2}-\overset{O}{\overset{\|}{C}}$),

3.65 (s, 3H, $O-\underline{CH_3}$), 4.5 (m, 1H, tertiary proton),

5.05 (s, 2H, $-O-\underline{CH_2}$ -Ph), 7.3 (m, 5H, aromatic).

XVII. Reaction of Z-Asn-OMe (5) with $PhI(TFA)_2$ followed by treatment with EtNCS : Isolation of (7a) and (8).

A stirred suspension of $PhI(TFA)_2$ (0.927g, 2.14 mmol), pyridine (0.25 ml, 2.8 mmol) in $CH_3CN:H_2O$::1:1 (14 ml) was admixed with, over 0.5 h, (5) (0.4g, 1.43 mmol). The reaction mixture was left stirred at rt for 3 h, concentrated, diluted with H_2O (20 ml), extracted with EtOAc (2x25 ml), dried, evaporated. The resulting crude (6) was used as such in all reactions with RNCS. A solution of (6) as prepared above, in CH_2Cl_2 (10 ml) was admixed with EtNCS (0.5 ml) + Et_3N(0.5 ml) was left stirred at rt, overnight, evaporated and subjected to preparative TLC using PhH:EtOAc::7:3 as developer to give 0.09 g (20%) of (8) mp. 108°C, and 0.18g (41%) of (7a) as thick oil.

(7a) : Anal : Calculated for $C_{15}H_{21}N_3O_4S$ (mol. wt.= 339)

C, 53.1; H, 6.19; N, 12.39;

Found : C, 52.8; H, 6.27; N, 12.38.

IR : ν_{max}(neat) cm^{-1}: 3440, 3340 (-NH), 3000, 1720 (ester), 1540 (amide), 1510.

NMR : $\delta(CDCl_3)$: 1.20 (t, 3H, $-CH_2-\underline{CH}_3$), 3.35(m, 2H, $-\underline{CH}_2-CH_3$), 3.7 (s, 3H, $-O\underline{CH}_3$), 3.95 (m, 2H, $-\underline{CH}_2-N-$), 4.45 (m, 1H, tertiary proton), 5.05 (s, 2H, $-O-\underline{CH}_2-Ph$), 7.3 (s, 5H, aromatic).

ms : m/z : 339 (M^+).

(8): Anal : Calculated for $C_{14}H_{17}N_3O_3S$ (mol. wt. = 307)

C, 54.73; H, 5.53; N, 13.68;

Found C, 54.27; H, 5.59; N, 13.02;

IR : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 3190,1710, 1560 (amide), 1530.

NMR : $\delta(CDCl_3)$: 1.2 (t, 3H, $-\underline{CH}_2-\underline{CH}_3$), 3.65-4.85(m, 5H, $-\underline{CH}_2-CH_3$, $-\underline{CH}_2-N$ and tertiary proton), 5.10 (s, 2H, $-O-\underline{CH}_2-Ph$), 7.3(s, 5H, aromatic).

$[\alpha]_D^{25} = -2.4^o$.

ms : m/z 307 (M^+).

XVIII Thermal cyclization of (7a → (8) :

A solution of (7a) (0.180g) in dry benzene (10 ml)

was refluxed for 6 h, evaporated and the residue on preparative TLC using PhH:EtOAc::7:3 as developer gave (8) and unchanged (7a) in nearly equal amounts.

XIX. Reaction of Z-Asn-OMe (5) with $PhI(TFA)_2$, followed by PhN=C=S treatment : Isolation of (7b).

Compound (6) prepared precisely as described in EXPERIMENT XVII, from (5) (1.43 mmol) was taken up in CH_2Cl_2 (20 ml), admixed with PhNCS (0.378g, 2.8 mmol) + Et_3N(1 ml), left stirred overnight and evaporated. Chromatography of the residue over silica gel using EtOAc::PhH::40:60 as eluent gave (7b),(0.378g, 63%), mp. 122°C.

7b : Anal: Calculated for $C_{19}H_{21}N_3O_4S$(mol.wt.= 387);

C, 58.91; H, 5.42; N, 10.85;

Found C, 58.42; H, 5.62, N, 10.32.

IR : ν_{max}(KBr) cm^{-1}: 3380, 3320, 3220 (-NH),

1730 (ester), 1605, 1555 (amide).

NMR : $\delta(CDCl_3)$, 3.7 (s, 3H, -O-CH_3), 4.1 (m, 2H, -CH_2-),

4.5 (m, 1H, tertiary proton), 5.0 (s, 2H, -O-CH_2-Ph),

7.25 (m , 10H, aromatic).

ms : m/z : 387 (M^+).

XX. Reaction of Z-Asn-OMe(5) with $PhI(TFA)_2$ followed by treatment with $C_6H_{11}N=C=S$: Isolation of (7C) .

Compound (6), prepared as described in EXPERIMENT XVII, from (5) (2.6 mmol) was taken up in CH_2Cl_2(20 ml), and admixed with cyclohexyl isothiocyanate (1 ml) + Et_3N(1 ml), left stirred overnight at rt and evaporated. Chromatography over silica gel (Eluent:PhH:EtOAc::60:40) gave (7C) (0.228g, 23%) mp. 126-28°C.

7C : Anal : Calculated for $C_{19}H_{27}N_3O_4S$ (mol. wt. = 393) :

C, 58.01; H, 6.87; N, 10.68;

Found C, 57.18; H, 7.06; N, 10.25.

IR : ν_{max}(KBr) cm^{-1}: 3370 (-NH), 2960, 2880, 1740 (ester), 1710, 1565, 1545 (amide);

NMR : $\delta(CDCl_3)$: 1.2-2.0 (m, 11H, cyclohexyl protons), 3.75 (s, 3H, -O-C<u>H</u>$_3$), 3.9 (m, 3H, -C<u>H</u>$_2$- and -N(H)-C(H)<), 4.40 (m, 1H, tertiary proton), 5.05(s, 2H, -O-C<u>H</u>$_2$-Ph), 7.3 (s, 5H, aromatic).

ms : m/z : 393 (M^+).

XXI. Preparation of benzyloxycarbonyl asparaginyl phenylalanine methyl ester (Z-Asn-Phe-OMe, 9).

a. Diphenyl phosphoryl chloride.

A mixture of freshly distilled phenol (150g, 1.6 mole) and $POCl_3$(82 ml, 0.53 mole) was cautiously heated to 180°C (inside temp.), held at that temperature for 2 h and fractionally distilled to give diphenyl phosphoryl chloride (66g, 30%), B.P. 150°/1mm (lit. B.P. 140 - 155°/1.3 mm).

b. Diphenyl phosphoryl azide.

A mixture of diphenyl phosphoryl chloride (15.1g, 56.5 mmol), sodium azide (4g, 56.5 mmol) and dry acetone (50 ml) was left stirred overnight at rt, filtered, washed with dry acetone (10 ml), and evaporated to give diphenyl phosphoryl azide (13.9g, 90%) which was used as such for the coupling reaction.

c. Z-Asn-Phe-OMe(9).

To an ice cooled and stirred mixture of Z-Asn (2.5g, 9.4 mmol) and Phe-OMe.HCl (2.5g, 4.57 mmol) was added diphenyl phosphoryl azide (3.2g, 12 mmol) followed by Et_3N (3.2 ml 23 mmol) in dry DMF (20 ml). The reaction mixture was left stirred under ice-cooling for 7-8 h, then overnight atrt, concentrated *in vacuo* diluted with H_2O (30 ml), the resulting solid filtered, washed with 1N HCl (30 ml), 10% $NaHCO_3$(30 ml), H_2O, dried and crystalised from hot EtOAc to yield 3.8g (95%) of Z-Asn-Phe-OMe,(9), mp. 198°C (lit. mp. 19 97°C).

IR : ν_{max}(KBr) cm^{-1}: 3440, 3300 (-NH), 1750 (ester), 1700, 1640, 1550 (amide).

NMR : $\delta(CDCl_3 + DMSO\text{-}d_6)$: 2.05 (d, 2H, $-C\underline{H}_2-\overset{O}{\overset{\|}{C}}-$), 3.0 (d, 2H, $-C\underline{H}_2-Ph$), 3.65(s, 3H, $-OC\underline{H}_3$),4.5 (m, 2H, tertiary protons), 5.0 (s, 2H, $-O-C\underline{H}_2-Ph$), 7.15 (s, 5H, aromatic), 7.3(s, 5H, aromatic).

ms : m/z : 427 (M^+).

XXII. Preparation of benzyloxycarbonyl- β -Cyano alanine (Z-Ala(β -Cyano)-OH).

To a stirred and ice-cooled solution of Z-Asn (1.5g, 5.6 mmol) in pyridine (7.5 ml) was added a solution of DCC (1.22g, 5.9 mmol) in pyridine (3.8 ml). The mixture was left stirred at rt for 3 h, concentrated in vacuo,diluted with H_2O (25 ml), filtered, the filtrate acidified using (2N HCl), the resulting solid collected and dried, to yield Z-Ala(β -Cyano) -OH (1.12g, 80%), mp 128°C (lit. mp. 132°C).

IR : ν_{max}(KBr) cm^{-1}: 3340 (-NH), 2300 ($-C\equiv N$),1750, 1700, 1550 (amide).

XXIII. Preparation of Z-Ala(β -Cyano)-OMe(10).

To an ice-cooled solution of Z-Ala(β -Cyano)-OH (1.12g, 4.5 mmol) in MeOH (10 ml) was added an ethereal solution of CH_2N_2-prepared from N-nitroso-methyl Urea (3g) and 20% KOH solution (15 ml). The ester was precipitated with hexane refrigerated overnight, filtered and dried to give 1.04g (88%) of α -(Z)- β -(Cyano)-Ala-OMe(10) mp. 89°C (lit.mp.92°C).

IR : ν_{max}(KBr) cm^{-1}: 3350 (-NH), 2290 (-C≡N),1750 (ester), 1700, 1560 (amide).

NMR : δ ($CDCl_3$) : 2.95 (d, 2H, -CH_2CN), 3.80 (s, 3H, -OCH_3), 4.55 (m, 1H, tertiary proton), 5.10 (s, 2H, -O-CH_2-Ph), 7.3(s, 5H, aromatic).

XXIV. Reaction of Z-Gln-OMe(1a) with $PhI(TFA)_2$ followed by treatment with t-BuOCl : Isolation of N-benzyloxy-carbonyl- β -Cyano alanine methyl ester (10).

A stirred suspension of $PhI(TFA)_2$ (3.56g, 5.5 mmol) in $CH_3CN:H_2O$::1:1 (30 ml) was admixed with (1a) (1.08g, 3.67 mmol) and then pyridine (0.59 ml, 7.34 mmol). The reaction mixture was left stirred for 3 h, rt, concentrated in vacuo, diluted with water (10 ml), extracted with EtOAc (2x50 ml), dried, evaporated,

the residue triturated with hot hexane (2x20 ml) to remove PhI, dissolved in MeOH (10 ml), cooled, then admixed with freshly prepared t-BuOCl (6.5g, excess)[49] followed by, after 0.25 h, excess pyridine, left stirred overnight at rt, evaporated, the residue dissolved in $CHCl_3$ (60 ml), washed with 1N citric acid (50 ml), 20% NaCl solution (25 ml), dried, evaporated and subjected to preparative TLC (using PhH:EtOAc::80:20, as developer) to give 0.29g of (10),(30%), mp. 88°C (lit. mp. 91°C).TLC and IR of the product was identical with that of authentic sample.

XXV. Preparation of N-(formamidomethyl) N-benzyl morpholinium-iodide (12).

a. N-Formamido methyl morpholine :

To stirred morpholine (36 ml, 400 mmol) and formamide (14 ml, 400 mmol) was added at rt, over 0.5 h, formalin (32 ml, 400 mmol). The reaction mixture was left stirred at rt for 12 h, concentrated in vacuo, saturated with NaCl, extracted with $CHCl_3$ (3x100 ml), dried and evaporated to yield N-formamidomethyl morpholine, (52g, 90%) which was used as such for alkylation.

b. Benzyl Iodide :

To a stirred solution of NaI (54g, 350 mmol) in dry acetone (200 ml) was added benzyl chloride (34.8 ml, 300 mmol). The reaction mixture was left stirred in dark overnight, filtered,

evaporated, washed with 20% solution of sodium thiosulphate, dried and distilled under reduced pressure to yield 57g (86%) of benzyl iodide. bp. 93°/10 mm. (HIGHLY LACHRYMATORY!)

c. N-(formamidomethyl)-N-benzylmorpholinium Iodide (12).

An ice-cooled and stirred solution of N-Formamidomethyl morpholine (21.88g, 152 mmol) in dry $CHCl_3$ (100 ml), was admixed with benzyl iodide (48g, 220 mmol) refrigerated (2 days) and filtered to give white crystals of (12), (39g, 71%) mp. 156°C.

IR : ν_{max} (KBr) cm^{-1}: 3380, 3210, 3050, 1715, 1685, 1545 1530.

XXVI. Reaction of transfer synthon (12) with 2-thionaphthol : Isolation of 2-thionaphthyl methyl formamide (13).

Under nitrogen and stirring a mixture of 2-thionaphthol (3.2g, 20 mmol), (12) (7.24g, 20 mmol) and Et_3N (2 ml, 20 mmol) in dry benzene (50 ml) was refluxed for 6h, cooled washed with cold 2N HCl (3x15, 45 ml), H_2O (50 ml), dried, evaporated and chromatographed over silica gel (EtOAc:PhH::1:1 as eluent) to give (13) (3.90g, 90%) mp. 63°C,

13 : Anal. Calculated for $C_{12}H_{11}NOS$ (mol wt. = 217),

C, 66.3; H, 5.07; N, 6.45;

Found: C, 65.94; H, 5.34; N, 6.04.

IR : ν_{max}(KBr) cm^{-1}: 3310 (-NH), 1680, 1530 (amide);

NMR : $\delta(CDCl_3)$ 4.5 (d, J=7 Hz) + 4.7 (d, J=7Hz), 2H, -S-C$\underline{H}_2$, in the ratio 7.5:92.5, possibly arising from HN-CHO π barrier, 6.23 (br, 1H, -N$\underline{H}$), 7.3-8.1 (m, 8H, aromatic).

ms : m/z : 217 (M^+).

XXVII. Dehydraton of (13), Preparation of 2-thionaphthyl methyl-isocyanide (11).

Under nitrogen and stirring a mixture of 2-thionaphthyl methyl formamide (13) (4.25g, 20 mmol), Et_3N (3.5ml, 20 mmol) Ph_3P (6.3g, 24 mmol) dry CCl_4 (2.0 ml) in dry $CHCl_3$ (50 ml) was refluxed for 8 h, cooled, solvent evaporated and chromatographed over silica gel. Elution with benzene gave (11), (3.15g, 80%), mp. 75°C.

11 : Anal. Calculated for $C_{12}H_9NS$ (Mol. wt. = 199).

C, 72.36; H, 4.52; N, 7.05;

Found: C, 72.59,H, 4.66 N, 6.87.

IR : ν_{max}(KBr) cm^{-1}: 2140 (-NC).

NMR : $\delta(CDCl_3)$, 4.6(s, 2H, -S-C<u>H</u>$_2$-), 7.4-8.1 (m, 7H, aromatic).

ms : m/z: 199 (M^+).

XXVIII. <u>Reaction of thiophenol with (12) : Isolation of Thiophenyl methyl formamide (14)</u>.

Under nitrogen and stirring a mixture of thiophenol (0.110g, 1 mmol), (<u>12</u>) (0.362g, 1 mmol) and Et_3N (0.2 ml, 2 mmol) in dry benzene (15 ml) was refluxed for 6 h, cooled, washed with water (10 ml), cold 1N HCl (10 ml), water (10 ml), dried, evaporated and subjected to preparative TLC using EtOAc:PhH::30:70 as developer to yield 0.120g (72%) of thiophenyl methyl formamide, (<u>14</u>) mp. 32°C (lit. mp. 32°C).

IR : ν_{max}(thin film) cm^{-1}: 3320 (-NH), 1690, 1540 (amide).

NMR : $\delta(CDCl_3)$: 4.6 (d, J=7Hz , 2H, -S-C<u>H</u>$_2$), 7.3 (m, 6H aromatic), 7.9 (br, 1H, -C<u>H</u>O).

XXIX. <u>Reaction of benzaldoxime with (12) in benzene/NEt_3: Isolation of O-formamido methyl benzaldoxime (15)</u>.

A stirred mixture of benzaldoxime (1.2g, 10 mmol),

(12), (3.6g, 10 mmol) and Et_3N (1.4 ml, 10 mmol) in dry benzene (30 ml) was refluxed for 5 h, solvents evaporated in vacuo, the residue washed with H_2O (25 ml), extracted with EtOAc (2x25 ml), dried, evaporated and chromatographed over silica gel. Elution with EtOAc:PhH::20:80 gave 1.10g (61%) of (15), mp. 60°C.

15 : Anal: Calculated for $C_9H_{10}N_2O_2$ (mol. wt. = 178);

C, 60.60 H, 5.61, N, 15.73;

Found: C, 60.12; H, 6.03; N, 15.34.

IR : ν_{max}(KBr) cm^{-1}: 3290 (-NH), 1660, 1520 (amide).

NMR : $\delta(CDCl_3)$: 5.22 (t, 2H, $-OC\underline{H}_2-NH$), 6.7 (br, 1H, -NH), 7.3-7.7 (m, 5H, aromatic), 8.1 (d,1H), 8.25 (s, 1H).

ms : m/z : 178 (M^+).

XXX. Reaction of benzaldoxime with (12) in MeOH/NaOMe : Isolation of (15).

To a stirred solution of NaOMe in MeOH prepared by addition of Na (0.23g, 10 mmol) to dry MeOH (35 ml) was added benzaldoxime (1.2g, 10 mmol) followed by(12) (3.6g, 10 mmol). The mixture was refluxed for 6 h, evaporated, admixed with AcOH(10 ml

extracted with ether (2x50 ml), washed with water, dried, evaporated and chromatographed over silica gel. Elution with PhH: EtOAc::1:1 gave 1.36g (76%) of (15),mp.60°C. The spectral data of the product isolated was identical to that obtained from EXPERIMENT XXIX.

XXXI. Hydrolysis of O-formamidomethyl benzaldoxime (15) with $TiCl_3$: Isolation of benzaldehyde 2,4 -DNP.

Under nitrogen and stirring a solution of (15) (0.178g, 1 mmol) in dioxane (2 ml) was added to 10% aq. $TiCl_3$ (2.5 ml),ammonium acetate (1 g) and 50% aq. CH_3COOH (0.4 ml). The reaction mixture was left stirred under nitrogen at rt, for 24 h, extracted with ether (2x15 ml), dried evaporated, the residue dissolved in EtOH (2 ml), admixed with 2,4-DNP reagent, boiled, cooled, filtered and dried to give benzaldehyde 2,4-DNP (65%), mp. 234°C (lit. mp. 236°C).

XXXII. Reaction of β -Naphthol with (12) in MeOH/NaOMe : Isolation of dimer (16).

To stirred solution of NaOMe-prepared from sodium (0.23g, 10 mmol) and dry methanol (50 ml) was added β -naphthol (1.44g, 10 mmol) and(12) (3.62g, 10 mmol). The mixture was refluxed for 4 h, solvent evaporated, washed with water (50 ml), and the residue chromatographed over silica gel. Elution with EtOAc:PhH::10:90 gave (16),(1.15g, 74%), mp. 196°C (lit. mp.200°C).

IR : ν_{max}(KBr cm^{-1}: 3360 (-OH), 1640, 1620,

NMR : $\delta(CDCl_3 + DMSO\text{-}d_6)$: 4.76 (s, 2H, $-C\underline{H}_2-$),

7.5-6.8 (m, 12H aromatic), 9.7 (s, 2H, -O<u>H</u>).

ms : m/z : 300 (M^+).

XXXIII. <u>Reaction of β -Naphthol with (12) in benzene/NEt_3: Isolation of 16</u>.

A stirred mixture of β-Naphthol (0.145g, 1 mmol), (<u>12</u>) (0.362g, 1 mmol) and Et_3N (0.15 ml, 1 mmol) in dry benzene (20 ml) was refluxed for 6 h, cooled, washed with water (20 ml), cold 2N HCl (20 ml), dried, evaporated, and subjected to preparative TLC using EtOAc:PhH::10:90 as developer to give0.06g,(40%) of (<u>16</u>), mp. 195°C. IR of this product was found to be identical to (<u>16</u>), that obtained from EXPERIMENT XXXI.

XXXIV. <u>Reaction of (12) with Indole : Isolation of dimer (17)</u>.

A solution of Indole (1.17g, 10 mmol) and (<u>12</u>) (3.62g, 10 mmol) in DMSO (30 ml) was left stirred at rt, for 24 h, diluted with water (250 ml) extracted with CH_2Cl_2(50 ml), dried, evaporated and chromatographed over silica gel. Elution with benzene gave unreacted indole (0.20g) and with EtOAc:PhH::

10:90, 0.35g (20%) of (17) mp. 162°C (lit. mp. 162°C).

IR : ν_{max}(KBr) cm^{-1}: 3420, (-NH), 1625, 1605,1460.

NMR : $\delta(CDCl_3)$: 4.2 (s, 2H, $-C\underline{H}_2$), 7.6-6.9 (m, 12H, aromatic)

ms : m/z : 246 (M^+).

XXXV. Reaction of phenol with (12) : Isolation of Mannich base (1

To stirred NaOMe -prepared from Na (0.23g,10 mmol) and dry MeOH (50 ml) was added PhOH (0.984g, 10 mmol) and (12) (3.62g,10 mmol). The mixture was refluxed for 8 h, solvents evaporated, the residue washed with water (50 ml), dissolved in EtOAc (20 ml), dried, evaporated and chromatographed over silica gel. Elution with EtOAc:PhH::10:90 gave 0.190g, (10%) of (18), mp.90°C.

IR : ν_{max}(KBr) cm^{-1}: 1625, 1600, 1500, 1470.

NMR : $\delta(CDCl_3)$: 2.5 (m, 4H, morpholine protons), 3.7 (m, 6H, morpholine protons), 6.6-7.4 (m, 4H aromatic), 8.3 (br, 1H, -OH).

ms : m/z : 193 (M^+).

XXXVI. Reaction of tetracyclone with (12) : Isolation of dihydrotetracyclone (19).

A stirred solution of tetracyclone (0.192g, 0.5 mmol)

and (12), (0.362g, 1mmol) in O-dichlorobenzene (10 ml) was refluxed for 24 h, evaporated in vacuo and chromatographed over silica gel. Elution with EtOAc:PhH::20:80 gave 0.120g (63%) of (19), mp. 160°C.

IR : ν_{max}(KBr) cm^{-1}: 3040, 3020, 1690, 1490, 1350.

NMR : $\delta(CDCl_3)$: 3.75 (d, J=7Hz, 1H), 4.55 (d,J=7Hz, 1H), 7.1-7.4 (m, 20H, aromatic).

ms : m/z : 386 (M^+).

XXXVII. Reaction of the conjugate base of 2-thionaphthyl methyl isocyanide(11) with acetonitrile : Preparation of 4-thionaphthyl, 5-methyl imidazole (20).

To a stirred solution, held at -78°C, of CH_3CN (1 ml) n-BuLi solution in hexane (10 ml, 5 mmol) in dry THF (5 ml) was added, over 0.25 h, a solution of(11)(1g, 5 mmol) in dry THF (5 ml). After 0.25 h at -78°C, the reaction mixture was allowed to come to room temperature and then left stirred for 0.5 h, solvents were evaporated, the residue triturated with water, filtered and dried to give 1.15g (95%) of (20), mp. 190°C.

20 : Anal. : Calculated for $C_{13}H_{12}N_2S$ (mol. wt. = 240):

C, 70.00; H, 5.00; N, 11.66.

Found : C, 69.62; H, 4.74; N, 11.28.

NMR : δ ($CDCl_3$ + DMSO-d_6) 2.25 (s, 3H, im-CH_3), 7.05-7.95 (m, 9H, aromatic).

ms : m/z: 240 (M^+).

XXXVIII. Reaction of the conjugate base of 2-thionaphthyl methyl isocyanide (11) with benzonitrile : Preparation of 4-thionaphthyl, 5-phenyl imidazle (21).

The crude cycloaddition product prepared from (11) (5 mmol) and PhCN (0.5 ml) precisely as described in EXPERIMENT XXXVII was extracted with hot hexane (2x25 ml) to remove 0.5g of unchanged (11). The residue was washed with MeOH (10 ml) to give compound (21), (0.480g, 64%) mp. 223°C.

21 : Anal. : Calculated for $C_{19}H_{14}N_2S$ (mol. wt. = 302);

C, 75.49; H, 4.63; N, 9.27;

Found : C, 75.06; H, 5.02; N, 9.06.

NMR : δ($CDCl_3$) : 7.2-8.05 (m, aromatic protons).

ms : m/z : 302 (M^+).

XXXIX. Desulfurization of 4-thionaphthyl-5-methyl imidazole (20) Isolation of 4(5) methyl imidazole (22).

A solution of (20), (1g, 4.12 mmol) in dry EtOH (100 ml) was left stirred for 5 h, at rt, with freshly prepar

W-6 Raney nickel (4-5g)[50], filtered,evaporated and the residue chromatographed over silica gel. Elution with MeOH:EtOAc::5:95 gave 0.3g (76%) of (22) mp. 45°C (lit. mp. 46°C).

NMR : $\delta(CDCl_3)$: 2.2 (s, 3H, im-CH_3), 6.7 (s, 1H, -NH), 7.4 (s, 1H, imidazole proton), 8.2 (s, 1H, imidazole proton).

XL. Desulfurization of 4-thionaphthyl-5-phenyl imidazole (21) : Isolation of 4, (5) phenyl imidazole (23).

Desulfurization of (21) (0.4 mmol) as described in EXPERIMENT XXXIX followed by preparative TLC using EtOAc as developer gave 0.032g, (56%) of (23), mp. 126°C (lit. mp. 129°C).

NMR : $\delta(CDCl_3)$: 6.2 (bs, 2H, -NH), 7.15-7.70 (m, 7H,aromati

XLI. Reaction of Z-Ala(β -cyano)-OMe(10) with conjugate base of (11) : Attempted preparation of histidine (24).

The cyclo-addition of (10) with the conjugate base of (11)-prepared as described in EXPERIMENT XXXVII, with an added equivalent of n-Buli to neutralize Z-NH-moiety gave a complex mixture from which pure products could not be isolated.

XLII. Reaction of lysine with basic cupric carbonate followed by treatment with 4-chloroquinazoline : Isolation of Q-Lys-Coppercomplex (26).

A mixture of lysine mono HCl (5g, 27 mmol) and basic cupric carbonate (9.04g) in H_2O (200 ml) was refluxed for 2h, filtered, admixed with solid $NaHCO_3$(8.8g), then with a solution of 4-chloroquinazoline (4-6g, 28 mmol) in acetone (120ml), left stirred at rt, for 2d, filtered, washed with MeOH (50 ml) and dried to yield 6.61g, (82%) of (26) mp. 210°C (d).

IR : ν_{max}(KBr) cm^{-1}: 3300, 1690, 1625, 1610.

XLIII. Decomplexation of (26) followed by reaction with benzyloxycarbonyl chloride and diazomethane : Isolation of Z-Q(Me)-Lys-OMe (29).

Compound (26), (6g, 20 mmol) was dissolved in 4N HCl (50 ml), a stream of H_2S passed till no more black precipitate was formed, filtered, neutralized with 4N NaOH, cooled in ice, treated batchwise with 9 ml of 50% benzyloxycarbonyl chloride in toluene and 4N NaOH making pH 8-9, acidified using cold 6N HCl, and the separated gummy solid dried. The crude Z-Q-Lys-OH (27) thus obtained (4g, 50 %) was dissolved in MeOH (25 ml), treated with ethereal diazomethane, evaporated and the residue chromatographed over silica gel. Elution with EtOAc:PhH::75:25 gave 1g (23%) of (29), mp. 140°C.

IR : ν_{max}(KBr) cm^{-1}: 3420, 3400 (-NH), 1740 (ester), 1720, 1660, 1560 (amide).

NMR : $\delta(CDCl_3)$: 1.65-2.1 (m, 9H, lysine $(C\underline{H}_2)_3$, Q-$C\underline{H}_3$), 3.7 (m, 5H, -O-$C\underline{H}_3$, Q-HN-$C\underline{H}_2$-), 5.05 (s,2H, O-$C\underline{H}_2$Ph), 7.25-7.75 (m, 9H, aromatic), 8.5 (s, 1H, quinazoline proton).

ms : m/z : 437 (M^+).

XLIV. Decomplexation of (26) followed by benzoylation : Isolation of Bz-Q-Lys-OH (28).

A solution of (26), (1.0g, 1.65 mmol) in 2N HCl (20 ml) was subjected to H_2S stream till no more black precipitate formed, filtered, neutralized with cold 4N NaOH solution and benzoylated as described in EXPERIMENT XLII. The crude product was crystalized from MeOH:Ether to give 0.515g (41%) of (28), mp. 185-87°C.

IR : ν_{max}(KBr) cm^{-1}: 3445, 3300 (-NH), 1750, 1650, 1550 (ami

NMR : δ(DMSO-d_6): 1.25-2.15 (m, 6H,-$(C\underline{H}_2)_3$-), 3.9 (m,2H, -N(H)-$C\underline{H}_2$-), 7.3-8.7 (m, 10H, aromatic),

ms : m/z : 378(M^+).

XLV. Reaction of lysine copper complex with Z-Cl followed by decomplexation : Isolation of ω -Z-Lys .

Lysine copper complex prepared from lysine mono HCl (3.64g, 20 mmol) and basic cupric carbonate (6.6g) as described in EXPERIMENT XLII, was stirred for 24 h at rt, with solid $NaHCO_3$ (6.5g) and 50% Z-Cl in toluene (7ml, 20 mmol). The resulting blue solid filtered, dried, dissolved in minimum 6N HCl, and treated with EDTA solution (2.5g , 8.5 mmol) in 4N NaOH (25 ml). Neutralization with 4N NaOH gave after filteration and drying ω-Z-Lys, (3.78g, 77%); mp. 250°C (lit. mp.250°C).

IR : ν_{max}(KBr) cm^{-1}: 3430 (br), 1620 (sh) (-COOH), 1590, 1520 (amide).

XLVI. Reaction of ω -Z-Lysine with benzoyl chloride followed by diazomethane : Preparation of α -Bz, ω -Z-Lys-OMe (30).

ω-Z-Lys-OH (3.7g, 13 mmol) was benzoylated as described in EXPERIMENT XLIII to yield 3.8g (77%) of α-Bz-ω-Lys-OH. This was dissolved in MeOH and treated with cold ethereal solution of freshly prepared diazomethane. Evaporation of solvent gave 3.5g (89%) of (30), mp. 68°C.

IR : ν_{max}(KBr) cm^{-1}: 3320 (-NH), 2970, 1760 (ester), 1710, 1650, 1550 (amide).

NMR : $\delta(CDCl_3)$: 0.9-2.15 (m, 6H, $(C\underline{H}_2)_3$ -),

3.35 (m, 2H, -NH-C$\underline{H}_2$-), 3.65 (s, 3H,-O-C$\underline{H}_3$),

5.0 (s, 2H, -O-C$\underline{H}_2$-Ph), 7.1-7.85 (m, 10H, aromat

XLVII. Oxidation of Quinazoline Lysine Copper Complex(26) with $FeCl_3$: Isolation of dibenzoyl lysine methyl ester (Di-Bz-Lys-OMe,(31).

Under stirring oxygen was passed for 24 h, at rt, through a solution of Q-Lys.Cu complex (26), (5g, 8.2 mmol) in 20% Aq AcOH (80 ml) admixed with $FeCl_3$ (2.75g, 17 mmol). Decomplexation followed by benzoylation and esterification, gave, as the sole isolable product di-Bz-Lys-OMe (13%). Blank experiments showed that Q-Lys bond is retained when oxygen was not passed.

31 : IR : ν_{max}(KBr) cm^{-1}: 3315 (-NH), 1725, 1630, 1570, 152

NMR : $\delta(CDCl_3)$: 1.4-2.2 (m, 6H, $(-C\underline{H}_2)_3$-), 3.4 (m, 2H, -NH-C$\underline{H}_2$-), 3.75 (s, 3H, O-C$\underline{H}_3$),4.75 (m, 1H, tertiary proton), 7.2-7.9 (m, 10H, aromatic).

ms : m/z : 368 (M^+).

SECTION F. REFERENCES

1. a. T.F. Spande, B. Witkop, Y. Degani and A. Patchornic, Adv.Protein Chem., 24, 97 (1970).

   b. A. Darbre,'Practical Protein Chemistry-A Handbook'. John Wiley & Sons, 67 (1986).

2. K. Katayama, L.H. Ericsson, D.N. Enfield, K.A. Walsh, H. Neurath, E.W. Davie, and K. Titani, Proc. Natl. Acad. Sci., U.S.A., 76, 4990 (1979).
3. J. Hermann, T. Titani, L.H. Ericsson, R.D. Wade, H. Neurath, and K.A. Walsh, Biochemistry, 18, 5672 (1979).
4. D.L. Enfield, L.H. Ericsson, K. Fujikawa, K.A. Walsh, H. Neurath and R. Titani, Biochemistry, 19, 659 (1980).
5. J.K. Blodgett, G.M. Loudon, and K.D. Collins, J. Am. Chem. Soc., 107, 4305 (1985).
6. A.S. Inglis, Methods. Enzymol., 91, 324 (1983).
7. A.F. Rosenthal and M.Z. Atassi, Biochim. Biophys. Acta, 147, 410 (1967).
8. O. Yonemitsu, T. Hamada, and Y. Kanaoka, Tet. Lett., 32, 3575 (1968).
9. K. Poulsen, K.J. Fraser, and E. Haber, Proc. Natl. Acad. Sci., U.S.A., 69, 2495 (1972).
10. M. Landon, Methods Enzymol.,47, 145 (1977).
11. M. Wakselman, E. Guibejampel, A. Raoult, and W.D.Busse, J. Chem. Soc. Chem. Commun., 21 (1976).
12. S. Otieno, Biochemistry, 17, 5468 (1978).
13. M. Morishita, T. Sowa, F. Sakiyama and K. Narita, Bull. Chem.Soc. Japan, 40, 632 (1967).
14. Y. Degani, A. Ratcharnik and J.A. Macloren, J. Am. Chem. Soc., 88, 3460 (1966).
15. Z. Bohak, J. Biol. Chem., 239, 2878 (1964).

16. E. Gross, Methods Enzymol., 11, 238 (1967).
17. E. Gross and J.L. Morell, Biochem. Biophys. Res. Commun., 59, 1145 (1985).
18. F. Sakiyama, M. Morishita, T. Sewa, and K. Narita, Bull. Chem. Soc. Japan, 39, 631 (1966).
19. K. Barlos, P. Mamos, D. Papaioannou, C. Sanida, and C. Antonopoulos, J. Chem. Soc., Chem. Commun., 1258 (1986).
20. I.J. Galpin and D.A. Hoyland, Tetrahedron, 41, 895 (1985).
21. J. Lenard and G.P. Hess, J. Biol. Chem., 239, 3275 (1964).
22. M. Wilchek, S. Sarid and A. Patchornik, Biochem. Biophys. Acta, 104, 616 (1965).
23. M.A. Ruttenberg, T.P. King and L.C. Craig, Biochemistry, 4, 11 (1965).
24. F.D'Angeli, E. Scoffone, F. Filira, and V. Giormani, Tet. Lett., 24, 2745 (1966).
25. F.D'Angeli, V. Giormani, F. Filira, and C. DiBello,Biochem. Biophys. Res. Commun., 28, 809 (1967).
26. T. Kaneko, I.Takeuchi and T. Inui, Bull. Chem. Soc., Japan, 41,974 (1968).
27. T. Kaneko, S. Kusumoto, T. Inui and T. Shiba, Bull. Chem. Soc., Japan, 41, 2155 (1968).
28. K. Iwai and T. Ando, Methods. Enzymol., 11, 263 (1967).
29. A. Fontana, W.E. Savige, and M. Zambonin, 'Methods In Peptide And Protein Sequence Analysis', Third Internat. Conf. Birr, C.(Ed.), Elsevier/North Holland, Amsterdam, New York, Oxford, 309 (1980).
30. A. Previero, M.A. Celetti-Previero and P. Jolles., Biochem. Biophys. Res. Commun., 22, 17 (1966).
31. M. Morishita, and F. Sakiyama, Bull. Chem. Soc., Japan, 43, 524 (1970).
32. S.C. Rall and R.D. Cole, J. Am. Chem. Soc., 92, 1800 (1970).
33. L.A. Cohen, and L. Farber, Methods Enzymol., 11, 299 (1967).
34. G.A. Rosenthal'Plant non-protein amino and imino acids', Academic Press, 1982.

35. Proceedings of the international conference on 'Genetic physico-chemical approaches for analysis of biological catalysis', Florence, Italy, 1986.

36. E.T. Kaiser and D.S. Lawrence, Science, 226, 505 (1984).

37. S. Wilkinsen, J. Chem. Soc. 104 (1951).

38. In the case of (1c), in addition to (3), the pyroglutamine was isolated in 18% yield.

39. In addition to (7c), an other product mp. 142°C, was isolated in this reaction whose structure could not be established.

40. J.P. Fournier and L.P. Bouthillier, J.Am.Chem.Soc., 74, 5210 (1952).

41. S. Ranganathan, D. Ranganathan and S.K. Singh, Tet.Lett.4955 (1985).

42. M. Kondo, K. Miyazaki, H. Kodama, and H. Horimoto, Bull. Chem. Soc., Japan, 58, 1171 (1985).

43. Including the most recent procedure (which became available to us after completion of the present work): ArSH + Tos-CH_2NHCHO-[DMF/NaH] → $ArSCH_2NHCHO$-[$POCl_3/Et_3N$] → $ArSCH_2NC$ (A.M. Van Leusen, J. Wildeman, J. Moskal and A.W.Van Hemert, Recl. Trav. Chim. Pays-Bas, 104, 177 (1985); Chem. Abstr., 104, 148443h (1986).

44. H. Bohme and G. Fuchs, Chem. Ber., 103, 2775 (1970).

45. Although the reactive intermediates leading to the formation of the dimers can be explained on the basis of products arising from formamidomethyl transfer to carbon followed by loss of elements of NHCHO, this process is considered unlikely, particularly since, no product arising from transfer were obtained. The yields were high and the fact that the isolation of (18), does support the formation of reactive intermediate (B).

46. M.R. Grimmett in Advanced in Heterocyclic Chemistry, Ed. A.R. Katritzky and A.J. Boulton, Vol. 27, p. 242, Academic Press, 1980.

47. A.M. Van Leusen and J. Schut, Tetrahedron Lett., 285 (1976)
48. C. Walsh 'Enzymatic Reaction Mechanisms', W.H. Freeman and Company, San Fancisco, p. 505, 1979.
49. Under protection from light, stirring and ice-cooling, a solution of NaOH (8g, 200 mmol) in H_2O (120 ml), was admixed with t-BuOH (7.4g, 100 mmol), and subjected to passage of chlorine for 1h. The resulting yellow layer was washed with cold, 10% Na_2CO_3 solution (4x25 ml), cold H_2O (50 ml), dried over anhydrous $CaCl_2$ and filtered to give t-BuOCl (6.5 g) which was used immediately for reaction.
50. Ni-Al alloy (25 g) was added in portions, during 0.5 h, to a stirred solution of NaOH (32 g) in water (120 ml), held at 50 $\pm$ 2°C. The mixture was left stirred at the same temperature for another hour, decanted, washed with distilled water (5x400 ml), 95% EtOH (3x50 ml) dry alcohal (50 ml), and used immediately for desulfurization.